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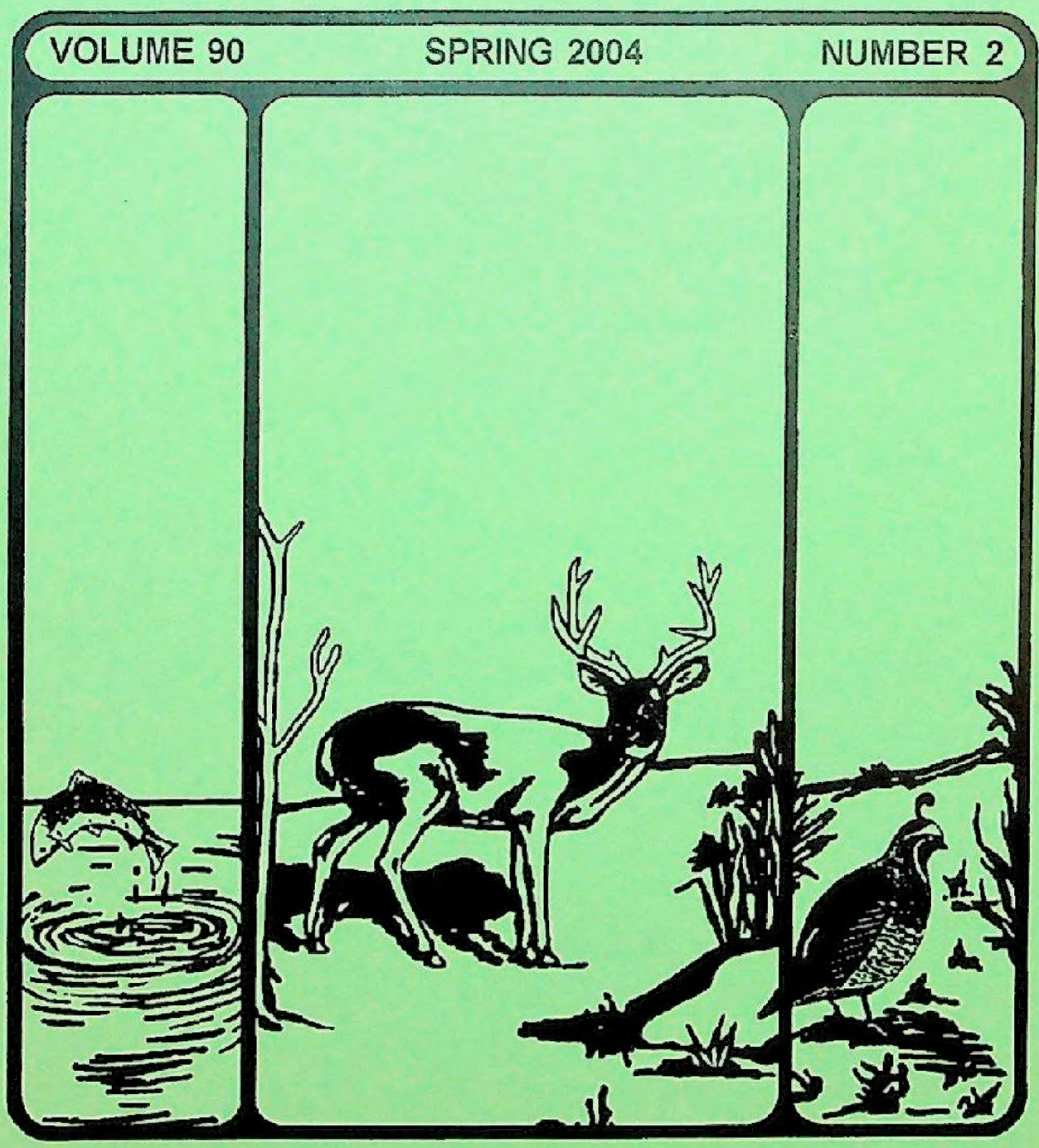

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Bleich, V. C., and B. M. Pierce. 1999. Expandable and economical long-term collars for juvenile mule deer. California Fish and Game 85:56-62.

In Table 1, the sample sizes for males and females fitted with Lotek transmitters should be 15 and 19, respectively, rather than 16 and 18.

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HARBOR SEAL, *PHOCA VITULINA RICHARDII*, POPULATION TRENDS IN THE SAN FRANCISCO BAY ESTUARY, 1970-2002

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Pacific harbor seals, *Phoca vitulina richardii*, have used the San Francisco Bay estuary in California as a nursery area and foraging site for thousands of years. Like other pinniped species, harbor seals in California were intensively hunted in the late 1800's and early 1900's, resulting in population declines obvious in the San Francisco Bay (SFB) by the 1920's. In 1972, the Marine Mammal Protection Act was passed, providing protection and management of harbor seal populations. We examined historical data (1970-1997) and our own ground counts at three primary SFB haulout sites (1998-2002) in order to understand the degree of recovery of the SFB harbor seal population. One of the largest estuaries on the west coast of the United States, SFB today is highly urbanized and heavily impacted by human activity. We documented a mixed response and recovery of harbor seals in SFB, likely due to a combination of factors, including habitat alteration, disturbance, pollution, and survey techniques. From 1970-2002, seal numbers at all three sites increased slightly during the fall/winter season, and increased at two sites during the pupping/molting season. At the largest SFB rookery site, however, no change was seen in seal numbers during the pupping/molting season.

INTRODUCTION

Along the west coast of North America, the Pacific harbor seal, *Phoca vitulina richardii*, ranges from Baja California to the Pribilof and Aleutian islands (Bigg 1969, Bigg 1981; Reeves et al. 1992). Periodically, harbor seals move onto offshore or intertidal rocks or reefs, sand bars, sandy beaches, or tidal mudflats in order to rest between

foraging trips, molt, thermoregulate or nurse their young (Bigg 1981, Allen[1] 1991, Watts et al. 1993). Seals tend to congregate at the same sites, called haulout sites, year after year (Fancher[2] 1979, Yochem et al. 1987, Harkonen 1987, Hanan[3] 1996). Such areas are characterized by ease of access to the water, proximity to food resources, and minimal disturbance levels (Scheffer and Slipp 1944, Loughlin[4] 1974, Allen[1] 1991). Although some haulout sites are used year-round by seals, others are used seasonally, for pupping, molting, or because of proximity to a seasonally-abundant prey resource (Risebrough et al.[5] 1980, Brown and Mate 1983, Slater and Markowitz 1986, Thompson 1989). Harbor seals often use haulout sites in bays and estuaries, particularly during the pupping and molting seasons, as estuaries provide sites protected from disturbance and sheltered from storms (Brown and Mate 1983, Harkonen 1987, Kopec and Harvey[6] 1995). Harbor seals generally feed on bottom-associated prey, in shallow waters of 30 to 50 m or less (Harkonen 1987; Harvey 1989, Tollit et al. 1998); estuaries provide large stretches of shallow bottom habitat, and can provide ample supplies of food (particularly small fish and crustaceans) for young seals (Bigg 1981, Harkonen 1987). In some areas, seals move seasonally and in large numbers into estuaries, following an abundant prey resource such as salmon or herring (Brown and Mate 1983, Allen[1] 1991, Greenstreet et al. 1993).

During pupping and molting seasons, harbor seals typically spend approximately 60% of their time on the haulout site (Yochem et al. 1987, Allen Miller[7] 1988, Harkonen and Heide-Jorgensen 1990; Thompson et al. 1997, Thompson et al. 1998). Counts of harbor seals made at haulout sites have been used to estimate population size, status and trends of harbor seals, often with correction factors derived from telemetry studies, designed to compensate for animals in the water at the time of survey and extrapolate haulout counts to population size (e.g. Hanan[3] 1996). Considerable research has been dedicated to determining the optimal time to survey haulout sites for maximum numbers

---

[1]Allen, S.G. 1991. Harbor seal habitat restoration at Strawberry Spit, San Francisco Bay. Report to the Marine Mammal Commission, Contract No. MM2910890-9, March 1991. 43 p.

[2]Fancher, L. 1979. The distribution, population dynamics and behavior of the harbor seal in South San Francisco Bay. M.S. Thesis. California State University, Hayward, CA. 109 p.

[3]Hanan, D. 1996. Dynamics of abundance and distribution for Pacific harbor seal (*Phoca vitulina richardsi*) on the coast of California. Ph.D. dissertation, University of California, Los Angeles. 158 pp.

[4]Loughlin, T.R. 1974. The distribution and ecology of the harbor seal in Humboldt Bay, California. M.S. Thesis, Humboldt State University, Humboldt, CA. 70 p.

[5]Risebrough, R.W., D. Alcorn, S.G. Allen, V.C. Anderlini, L. Booren, R.L. DeLong, L.W. Fancher, R.E. Jones, S.M. McGinnis, and T.T. Schmidt. 1980. Population biology of harbor seals in San Francisco Bay, California. Nat. Tech. Inf. Serv. Report No. MMC-76/19.

[6]Kopec, A.D. and J.T. Harvey. 1995. Toxic pollutants, health indices, and population dynamics of harbor seals in San Francisco Bay, 1989-1992. Moss Landing Marine Laboratories, P.O. Box 450, Moss Landing, CA USA 95039-0450

[7]Allen Miller, S. 1988. Movement and activity patterns of harbor seals at the Point Reyes Peninsula, California. M.S. Thesis, University of California at Berkeley (Wildland Resource Science), Berkeley, CA. 69 p.

(Fancher and Alcorn 1982, Allen et al. 1989, Thompson et al. 1989, Thompson et al. 1997, Frost et al. 1999). However, given the differences in seal site use by location, season, year, time of day, tide height, weather, and levels of disturbance (Paulbitski 1975, Stewart[8] 1981, Slater and Markowitz 1983, Allen et al. 1984, Pauli and Terhune 1987, Grellier et al. 1996, Frost et al. 1999), optimal survey time may vary from site to site (Thompson et al. 1997, Grigg et al. 2002). Generally, coastal sites are surveyed during the molt (Thompson et al. 1989), when seals tend to spend more time onshore, possibly to facilitate the molting process (Feltz and Fay 1966). To assess seal numbers in an estuarine habitat, haulout sites may be best surveyed during the pupping season (Thompson et al. 1997), given the apparent preference of estuarine sites for pupping (Harkonen 1987).

An observed decline in numbers of seals using a haulout site can be indicative of an actual population decline (for example, due to overhunting, decreased reproduction or survival) (Bartholomew and Boolootian 1960, Bonner et al. 1973). However, changes in haulout site numbers may be influenced by a number of factors, including seasonal differences in amount of time spent on the haulout site, or shifts away from one site to another. For example, adult female seals spend more time on the haulout site during the pupping season and, after molting, seals appear to spend more time in the water foraging (Thompson et al. 1989, Thompson et al. 1997). Seals have been reported to abandon a haulout site due to high levels of disturbance (Paulbitski 1975, Allen[1] 1991) or shift site use following shifts in prey abundance or location (Brown and Mate 1983, Thompson et al. 2001). Determining the cause or causes of observed shifts in seal numbers in a given area can be difficult, because such shifts can be due to a number of confounding factors.

The San Francisco estuary, more commonly called the San Francisco Bay (SFB), is the largest coastal embayment on the west coast of the United States (Conomos et al. 1985) (Fig. 1). Based on evidence from shellmounds found along the SFB shoreline, harbor seals have used this estuary for thousands of years (Nelson 1909, Broughton 1999). Limited data are available on seal numbers prior to the late 1800's (Scammon 1874, Bartholomew 1967), and reliable census data on harbor seals in SFB are almost non-existent prior to 1949 (Bartholomew 1949). The historical data suggest that seals were numerous in SFB prior to the late 1800's, but were hunted intensely in SFB and along the California coast during the late 1800's and early 1900's (Scammon 1874, Bonnot 1928, Bonnot 1951, Bartholomew and Boolootian 1960, Hildebrandt and Jones 1992), resulting in severe population declines that were obvious in SFB by the 1920's (Bonnot 1928). In the United States, marine mammals have been legally protected from hunting since 1972. Since that time, harbor seal numbers on the coast of California have increased markedly (Allen et al. 1989, Hanan[3] 1996, Sydeman and Allen 1999, Caretta et al.[9] 2001). Based on counts of harbor seals during aerial surveys of haulout sites conducted in May/June 1995, and a correction factor of 1.3, the California population

[8]Stewart, B.S. 1981. Seasonal abundance, distribution and ecology of the harbor seal (Phoca vitulina richardsi) on San Miguel Island, California. MS Thesis, San Diego State University, San Diego, CA. 66 p.

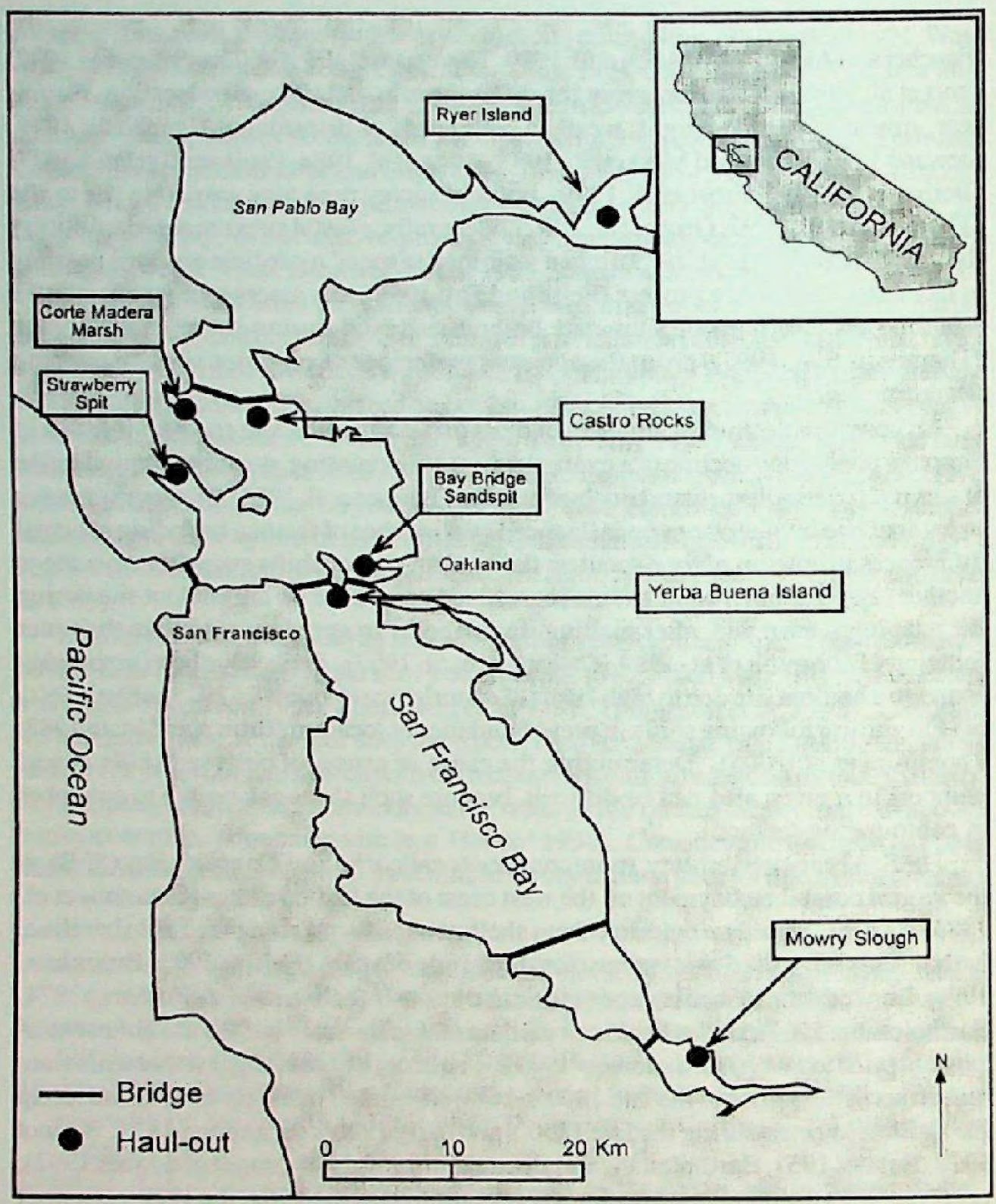

Figure 1: Map of San Francisco Bay harbor seal haulout sites. Shown are three primary haulout sites (Castro Rocks, Yerba Buena Island, and Mowry Slough), two smaller sites (Ryer Island, Corte Madera Marsh) and two historic sites (Strawberry Spit, Bay Bridge Sandspit).

is estimated at 30,293 (Caretta et al.[9] 2001).

Since the 1970's, considerable research has been conducted on harbor seals in SFB. The SFB population has been described as "stable" (Fancher[2] 1979, Risebrough et al.[5] 1980, Alcorn and Fancher[10] 1980, Fancher and Alcorn 1982), although some researchers have expressed concern about the loss of certain haulout sites, due primarily to disturbance (Bartholomew 1949, Paulbitski 1975, Allen[1] 1991). Since 1982, the California Department of Fish and Game (CDFG) has conducted statewide aerial surveys to assess population numbers of harbor seals in California; haulout sites in SFB are included in their surveys (Fluharty[11] 1999, Read and Sweetnam[12] 2002). Although the CDFG aerial surveys were not designed for evaluating population trends in SFB, their data for all SFB haulout sites have been cited as an indicator of lack of population increase in SFB (Kopec and Harvey[6] 1995). The CDFG aerial surveys support a population estimate of approximately 500 seals for SFB (R. Read, California Department of Fish and Game, personal communication). Recently, some SFB researchers have expressed concern about the lack of increase in harbor seal numbers in SFB, as these numbers do not appear to be rebounding compared to seal populations on the California coast (Kopec and Harvey[6] 1995). SFB is surrounded by three major urban areas and is home to a major west coast shipping port, with a rapidly expanding human population and a shoreline extensively altered by human activity. An estimated 94% of tidal marshes in SFB (i.e., estuarine habitat preferred by harbor seals) have been altered by filling or diking (Josselyn and Buchholz[13] 1984). Contaminant levels in the tissues of SFB seals have been found to be high (Risebrough et al.[5] 1980, Young et al. 1998, She et al. 2002), prompting concerns about possible negative effects on seal reproductive rates.

There are currently 20 active harbor seal haulout sites in SFB, although most are used sporadically or by small numbers of seals (<15) (Kopec and Harvey[6] 1995). We have been intensively surveying the three largest harbor seal haulout sites in SFB (Castro Rocks, Yerba Buena Island, and Mowry/Newark Sloughs) since 1998. This report:

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[9]Caretta, J.V., J. Barlow, K. A. Forney, M.M. Muto, and J. Baker. 2001. US Pacific Marine Mammal Stock Assessments: 2001. US Department of Commerce, NOAA Technical Memorandum NOAA-TM-NMFS-SWFSC-317.

[10]Alcorn, D.J. and L.E. Fancher. 1980. Report on the harbor seals of the San Francisco Bay National Wildlife Refuge, south San Francisco Bay, California. Final Report to the USFWS, SFBNWR. 30 April 1980.

[11]Fluharty, M.J. 1999. Summary of Pacific harbor seal, *Phoca vitulina richardsi*, surveys in California, 1982-1995. California Department of Fish and Game, Marine Region. Administrative Report 99-1. 49 p.

[12]Read, R. and D. Sweetnam. 2002. Final Report: Survey of Harbor Seals in California 2002. California Department of Fish and Game, Marine Region. 12 p.

[13]Josselyn, M. and J. Buchholz. 1984. Marsh restoration in San Francisco Bay, a guide to design and planning. Tiburon, CA: Romberg Tiburon Center for Environmental Studies. Technical Report no. 3. 103 p.

1. analyzes seasonal patterns of site use at these three important haulout sites, and
2. evaluates trends in harbor seal numbers at these three sites, in light of our data and available historical counts.

STUDY AREA AND METHODS

Castro Rocks (CR) is a rocky outcrop located in northern SFB, adjacent to the southeastern edge of the Richmond-San Rafael Bridge (Fig. 1). The rocks span approximately 265 m, beginning 17 m from the bridge and ending 75 m from the bridge. CR is the largest harbor seal colony in northern SFB (Fancher[14] 1987) and is the second largest pupping site in SFB (Kopec and Harvey[6] 1995). Seals haul out year-round on CR during medium to low tides, during the daytime and nighttime (Green et al.[15] 2002, Grigg et al. 2002).

Yerba Buena Island (YBI) is located at the midpoint of the San Francisco-Oakland Bay Bridge in central SFB (Fig. 1). The site is a cobblestone beach backed by a steep, 15-25 m high cliff. It is located on the south side of the island, approximately 3 km northeast of the San Francisco city shoreline, 2 km west of the Port of Oakland and approximately 650 m from the Bay Bridge. Harbor seals haul out on YBI year-round, under a wide range of tidal conditions, with maximum counts reported during the winter months when Pacific herring, *Clupea harengus pallasii*, spawn in SFB (Spencer16 1997, Green et al.[15] 2002). Although YBI is not historically known as a pupping site (Spencer[16] 1997), more recent data indicates that several pups are born here each year (Green et al.[15] 2002).

Mowry Slough (Fig. 1) and adjacent Newark Slough are located in the extreme south of SFB, on National Wildlife Refuge land. Given the proximity and similar seasonality of seal use of these two sloughs, seals counted at both were included in one total, and the area was treated as one site, Mowry/Newark sloughs (MNS). Seals at this site haul out at varying tides on sloping mud banks bordering the sloughs, backed by tidal marsh vegetation, and (at low tides) on mudflats at the slough mouths. MNS is the largest harbor seal rookery in SFB, and has a highly seasonal pattern of use, with high numbers during the pupping and molting seasons and markedly lower numbers of seals present during the fall and winter (Fancher[2] 1979, Alcorn and Fancher[10] 1980, Kopec and Harvey[6] 1995, Green et al.[15] 2002).

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[14]Fancher, L. 1987. An update on the current status of the harbor seal in San Francisco Bay. The Bay Institute of San Francisco, Sausalito, CA. February 1987.

[15]Green, D.E., E. Grigg, S. Allen, and H. Markowitz. 2002. Monitoring the potential impact of the seismic retrofit construction activities at the Richmond-San Rafael Bridge on harbor seals (*Phoca vitulina*): May 1998-December 2002 (Final Draft Interim Report, December 2002). Richmond Bridge Harbor Seal Survey, San Francisco State University (Biology), San Francisco, CA.

[16]Spencer, C.L. 1997. Seasonal haul-out patterns of *Phoca vitulina richardsi* in San Francisco Bay. M.A. Thesis, San Francisco State University, San Francisco, CA. 98 p.

Survey Methods (1998-2002)

We surveyed all three sites from May 1998 through December 2002. Surveys were conducted 3-7 days/week at CR, and 2 days/week at YBI and MNS. Surveys at CR and YBI were centered around the low tide when possible, when maximum daily numbers of seals are recorded at these sites (Green et al.[15] 2002). Surveys at MNS were centered around the low tide during pupping/molting seasons, and approximately 3-4 hours after the high tide at other times of the year, in accordance with information from previous studies (Fancher[2] 1979, Fancher and Alcorn 1982) on timing of maximum seal numbers at MNS.

Statistical Analyses: Long-term Trends (1970-2002)

In order to minimize impacts on our counts by disturbances which caused seals to leave the site, we used maximum counts for all comparisons. For the trends analysis, in order to maximize comparability to previous studies in SFB, we examined maximum counts from two "seasons": pupping/molting season (March through July), and fall/winter season (August through February). The maximum count for the trends analysis was defined as the maximum count (i.e., the maximum number of seals present at one time on the haulout site) obtained at a given site during the season noted. We evaluated current data against historical harbor seal counts for the three largest SFB haulout sites, using only data from 1970 onwards (Paulbitski[17] 1972; Paulbitski and Maguire 1972; Paulbitski[18] 1976; Fancher[2] 1979; Risebrough et al.[5] 1980; Alcorn and Fancher[10] 1980; Fancher and Alcorn 1982; Fancher[14] 1987; Allen[1] 1991; Kopec and Harvey[6] 1995; D. Kopec, personal communication; Green et al.[15] 2002). Not enough information was available on counts made prior to 1970 to assess the reliability of those counts. Data for these three sites was the most consistent source of data for trends in SFB; other SFB sites were surveyed only infrequently and were not included in the historical analysis.

In order to look for trends in seal numbers in SFB, we ran regressions on the natural logarithm of maximum harbor seal count (1970 – 2002) versus year, for the two seasons, for all sites. As we were not attempting to obtain absolute estimates for the SFB population as a whole, no correction factor was used on counts data prior to trends analysis. We calculated mean annual finite growth rate, G, from the slope of the regression line, m, as follows (Olesiuk et al. 1990, Hanan[3] 1996):

$$G = e^{m} - 1$$

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[17]Paulbitski, P.A. 1972. The adaptive hauling behavior and population variance of harbor seals, *Phoca vitulina richardi*, which haul-out at Strawberry Spit, Richardson Bay. Unpublished manuscript, San Francisco State University, January 1972.

[18]Paulbitski, P.A. 1976. Unpublished data, cited in Kopec, A D. and Harvey, J.T. (1995) Toxic pollutants, health indices, and population dynamics of harbor seals in San Francisco Bay, 1989-1992. Moss Landing Marine Laboratories, P.O. Box 450, Moss Landing, CA USA 95039-0450

Maximum seal counts do not include pups; whenever such data were available, we included as a separate category maximum number of pups counted each year, at each site.

In order to test whether long-term trends in seal numbers differed by site, we compared the slopes of the regression lines (natural logarithm of maximum count versus year) for the three sites, using Analysis of Covariance methods as described in Zar (1996).

Statistical Analyses: Recent Data (1998-2002)

For our recent surveys (1998-2002), we ran further analyses to evaluate possible trends revealed in the intensive survey efforts. For each site, we used Kruskal-Wallis nonparametric ANOVAs to determine whether maximum daily counts at each site differed by year. Since we began our surveys in May 1998, we ran these whole year comparisons on 1999-2002 only. In addition, we used Mann-Whitney U tests on maximum daily counts for each site to determine whether significant differences existed in seal site use by season (pupping/molting versus fall/winter). We used Kruskal-Wallis tests to evaluate whether differences existed between years in the number of seals using each site during a given season. When running seasonal comparisons, we only included data from years for which we had a complete season of data; for the pupping/molting season we used data from 1999-2002, for fall/winter we used data from 1998-2001. Maximum daily counts for the 1998-2002 analyses were defined as the maximum number of seals counted at a given site on each day surveyed. All statistical analyses, including regressions, were considered significant at $\alpha = 0.05$.

We calculated pup production for each year as the maximum number of pups counted during the pupping season, divided by the maximum number of adult/subadult seals counted during the same season, multiplied by 100. We then calculated a mean pup production rate for years 1999-2002, for all three sites.

Statistical Analyses: Aerial Survey Data (1982-2002)

Finally, in order to determine whether trends existed in the CDFG aerial survey data (1982-2002), and to compare these data with the counts from SFB researchers (Paulbitski[17] 1972; Paulbitski and Maguire 1972; Paulbitski[18] 1976; Fancher[2] 1979; Risebrough et al.[5] 1980; Alcorn and Fancher[19] 1980; Fancher and Alcorn 1982; Fancher[14] 1987; Allen[1] 1991; Kopec and Harvey[6] 1995; D. Kopec, personal communication; Green et al.[15] 2002), we ran a simple linear regression on the natural logarithm of the CDFG counts for all SFB haulout sites combined, and on the summed CDFG counts for the three sites featured in this paper: CR, YBI and MNS. CDFG surveys for SFB were not completed in 1997-1999, so no data were available for those years.

RESULTS

Long-term Trends (1970-2002)

Seasonal counts at CR during 1970-2002 revealed a steady increase in the number of adult and subadult harbor seals using that site, both during the pupping/molting season (r^2=0.78, P<0.001: mean annual growth rate: 6.1%) and during the fall/winter season (r^2=0.79, P<0.001; mean annual growth rate: 5.3%) (Fig. 2). A slight increase was also seen in number of pups counted at CR from 1970-2002 (r^2=0.77, P<0.001) (Fig. 2).

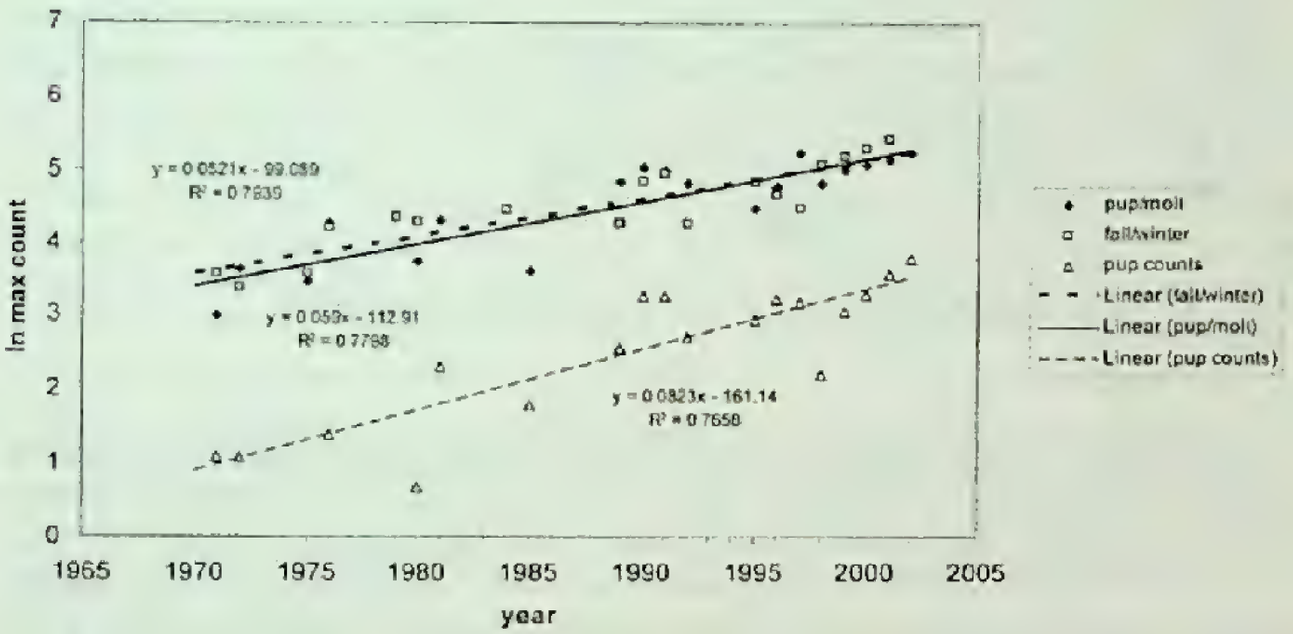


Figure 2: Castro Rocks maximum harbor seal counts (1970-2002)

YBI is a newer haulout site for SFB seals. Data available for YBI prior to 1980 suggest that few if any seals used this site in those years; no seals were present during a pupping/molting season count in 1970 (Paulbitski[18] 1976). Only 10 seals were counted at YBI in fall/winter season count in 1980, and no pups had been reported at YBI prior to that date by U.S. Coast Guard personnel living nearby (Fancher[14] 1987). For this reason, we found that a linear regression was not suitable for the 1970-2002 YBI data.

Given the paucity of data prior to 1989, we examined trends and growth rates for both seasons, and number of pups seen, for only those years when more consistent data were available for YBI (1989-2002). Based on linear regressions of the natural logarithm of maximum seal count by season since 1989, numbers of adult/subadult seals at YBI during the pupping/molting season did increase over the years (r^2=0.81, P<0.001; mean annual growth rate: 9.0%) (Fig. 3). There was no significant change in numbers during the fall/winter season (r^2=0.02, P>0.68). As with adult/subadult numbers during the pupping season, the number of pups counted each year at YBI also increased from 1989-2002 (r^2=0.87, P<0.001) (Fig. 3), although pup counts at this site remained low, with a maximum of 9 pups in 2001.

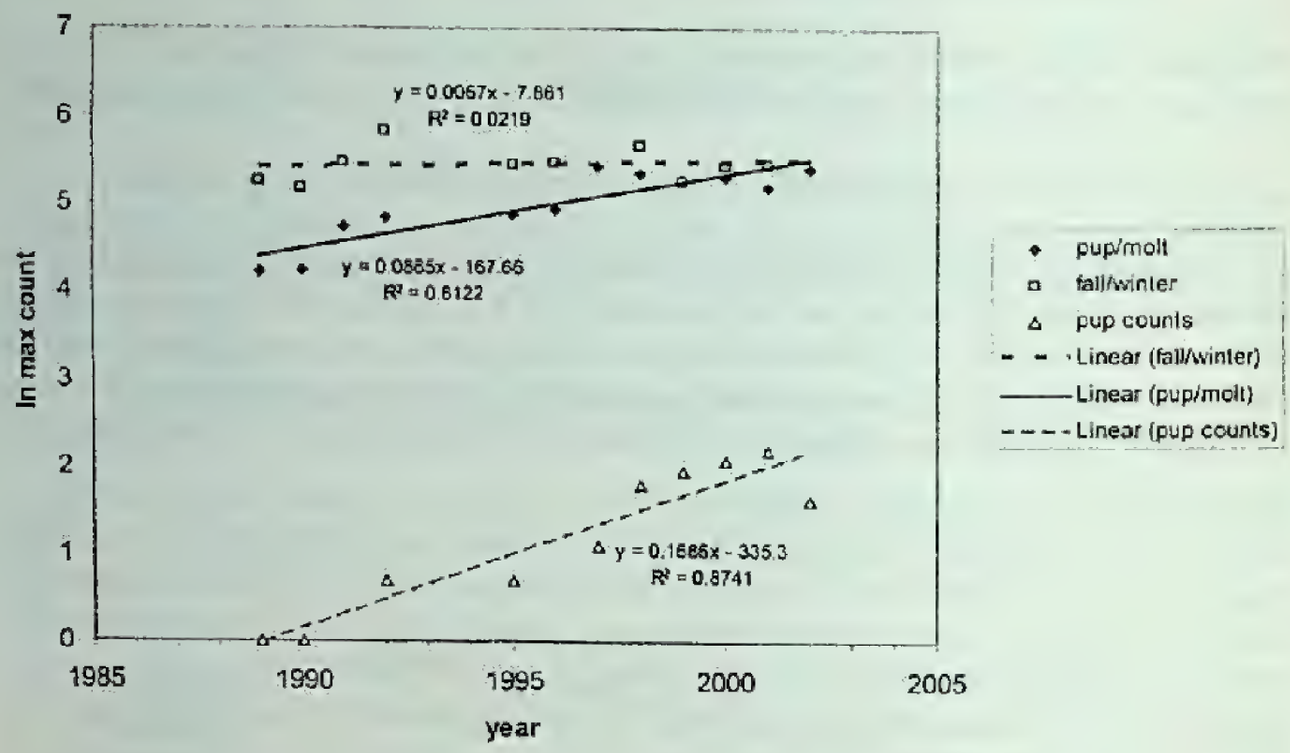


Figure 3: Yerba Buena Island maximum harbor seal counts (1989-2002)

No change was seen at MNS from 1970-2002 during the pupping/molting season ($P>0.05$) (Fig. 4). There was an increase in the number of seals during the fall/winter ($r^2=0.42$, $P<0.005$; mean annual growth rate: 3.4%). Two pup counts (1996 and 1998) were removed from the MNS analysis due to low sample size (1996: n=1, 1998: n=2); there was a very slight increase in the number of pups counted at MNS from 1970-2002 ($r^2=0.21$, $P<0.05$) (Fig. 4).

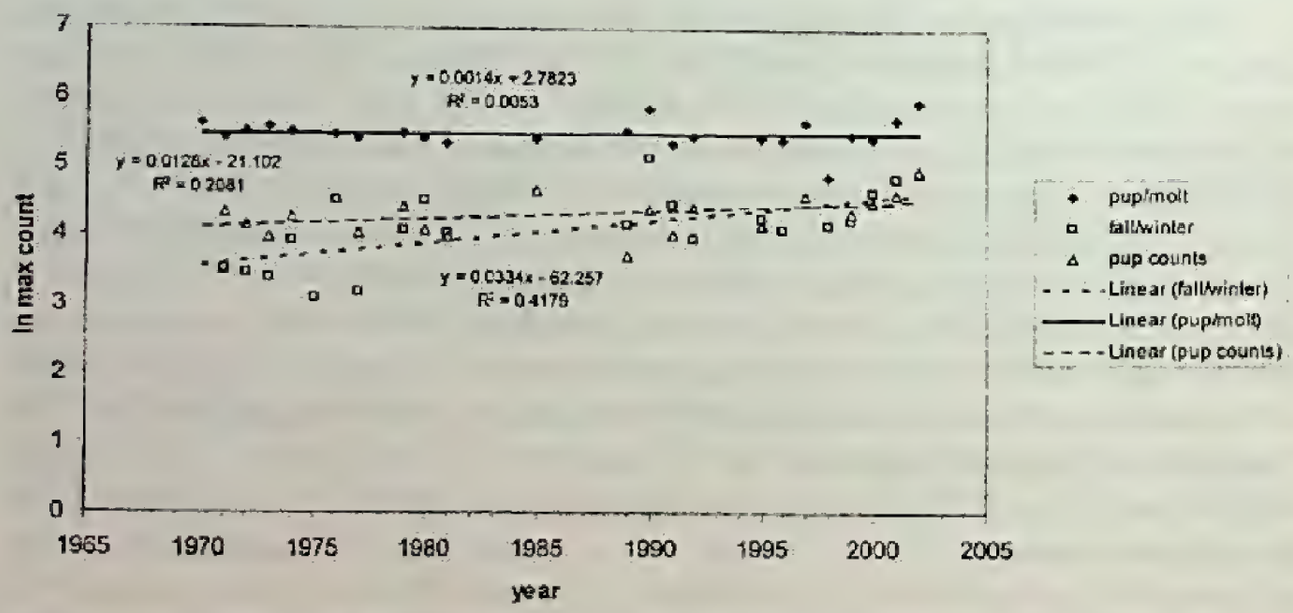


Figure 4: Mowry Slough maximum harbor seal counts (1970-2002)

The slopes for the three site regression lines were significantly different ($F_{(2, 97)}$ = 7.6168, P<0.001), further illustrating the differences in long-term trends in seal numbers at these three sites.

Recent Data (1998-2002)

In the comparisons of seal numbers across years at each site in the recent data, there was a significant difference in the number of seals using CR by year (H=16.34, df=3, P<0.01). A steady increase was seen in seals using CR in recent years, mirroring the yearly trend since 1970. There was no significant difference in the numbers of seals using YBI in recent years (H=4.33, df=3, P>0.22), although there was a drop in mean maximum count in 2002 (Table 1). There was a significant difference seen in counts at MNS across years (H=16.49, df=3, P<0.01), with little difference seen in the 1999-2000 counts, followed by a marked increase in numbers counted in 2001-2002 (Table 1).

In comparing seasonal use at each site for the recent data (1999-2002), numbers at CR were significantly higher during the pupping/molting season than during the fall/winter, for all years (1999: W=23423.5, P<0.001; 2000: W=24561.5, P<0.001; 2001: W=23012.5, P<0.001) (Table 1). At YBI, numbers were significantly higher during the pupping/molting season for two years, 1999 (W=5156.5, P<0.01) and 2000 (W=3227.5, P<0.05). Although numbers were slightly higher during the pupping/molting season for 2001, the difference between seasons was not significant (W=3408.5, P>0.21) (Table 1). At MNS, numbers were significantly higher during the pupping/molting season than during the fall/winter season, for all years (1999: W=10952, P<0.001; 2000: W=8691.0, P<0.001; 2001: W=5776.5, P<0.001) (Table 1).

A steady increase was seen across years (1999-2002) in numbers of seals using CR during the pupping/molting season (H=26.21, df=3, p<0.001) (Table 1). No significant differences were seen across years (1998-2001) in number of seals at CR during the fall/winter season (H=1.13, df=3, p>0.77). At YBI, no significant differences were seen across years in numbers of seals using this site during the pupping/molting seasons (H=0.76, df=3, P>0.85) (Table 1). However, a significant difference was seen in the number of seals at YBI during the fall/winter season (H=14.39, df=3, p<0.005), with numbers in 1998 markedly lower than other years. At MNS, there was a significant difference in numbers of seals present during the pupping/molting season (h=14.41, df=3, P<0.005), with increases seen in 2001 and 2002 counts (Table 1). There was a slight increase across years in the number of seals counted at MNS during the fall/winter, but the differences were not statistically significant (H=7.45, df=3, P>0.05).

Pup production rates at CR for 1999-2002 ranged from 14.0% to 26.5% (with rate increasing each year), with a mean of 19.4% ± 2.70% SE for the four years. At YBI, a site not previously considered a pupping site, pup production rates ranged from 3.1% to 6.3%, with a mean of 5.1% ± 0.70% SE; rate did not increase across years. At MNS, the largest rookery in SFB, pup production rates ranged from 32.1% to 39.1%, with a mean of 35.7% ± 1.60% SE; rate did not increase across years.

Table 1: Summary of harbor seal counts by year and season, 1998-2002: A) yearly mean of daily maximum counts, and n; B) seasonal mean and range of daily maximum counts; P/M = pupping/molting season (March-July); F/W = fall/winter season (August-February)

A)

Mean ± SE by year, and n

	1998[a]	1999	2000	2001	2002
Castro Rocks	52.7 ± 2.88	65.7 ± 2.77	70.3 ± 3.02	75.2 ± 3.32	80.4 ± 4.23
	n=199	n=280	n=287	n=274	n=255
Yerba Buena Island	56.7 ± 4.55	91.0 ± 4.67	83.3 ± 4.36	89.3 ± 4.93	78.3 ± 4.94
	n=108	n=129	n=102	n=99	n=109
Mowry Slough	36.9 ± 3.78	50.7 ± 3.67	53.6 ± 4.41	77.6 ± 6.54	75.3 ± 6.62
	n=89	n=165	n=144	n=114	n=125

[a]5/1/98 to 12/31/98 only

B)

Mean ± SE and (range) by season

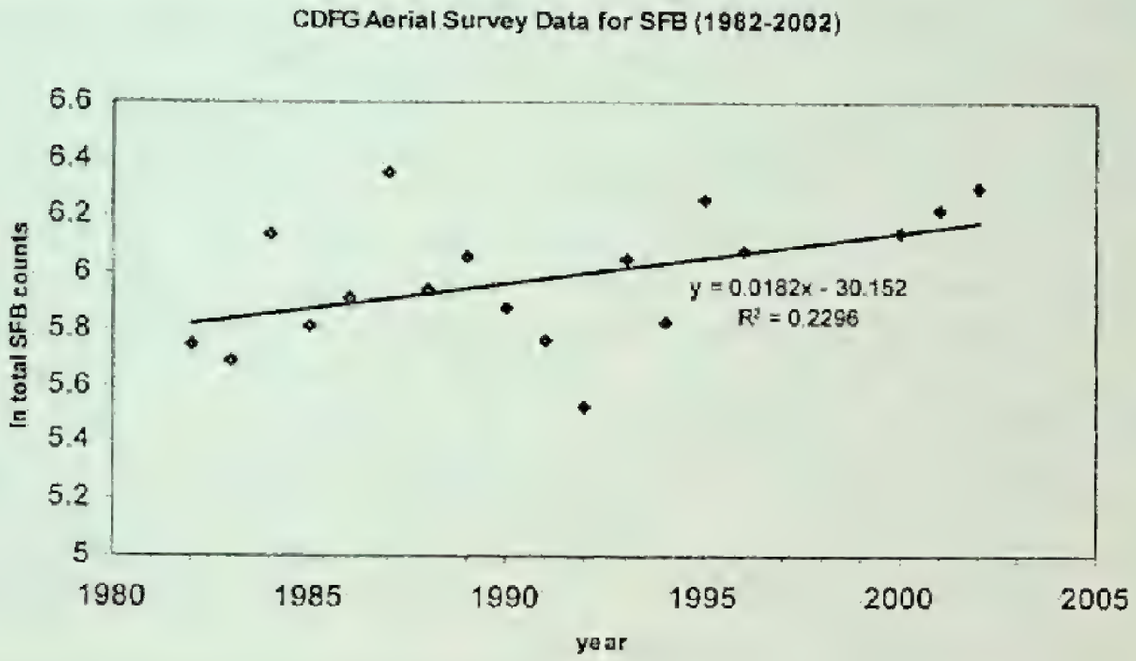
Year:	1998		1999		2000		2001		2002	
Season:	*P/M*[b]	*F/W*	*P/M*	*F/W*	*P/M*	*F/W*	*P/M*	*F/W*	*P/M*	*F/W*[c]
Castro Rocks	68.3 ± 4.51	51.4 ±3.14	86.7 ± 3.71	54.9 ± 3.56	96.6 ± 3.58	56.2 ± 3.59	107.3 ± 4.25	64.5 ± 4.70	117.5 ± 5.20	55.7 ± 5.96
	(16-121)	(0-160)	(12-150)	(0-179)	(12-161)	(0-201)	(6-172)	(0-225)	(9-200)	(0-226)
Yerba Buena Island	99.5 ± 8.93	57.9 ± 5.72	98.6 ± 7.57	80.6 ± 4.59	93.5 ± 6.77	76.9 ± 6.10	95.0 ± 7.40	86.2 ± 5.94	96.6 ± 7.10	49.7 ± 6.71
	(22-213)	(0-296)	(3-198)	(7-193)	(4-204)	(0-231)	(16-277)	(10-238)	(12-226)	(0-206)
Mowry Slough	79.6 ± 8.17	23.4 ± 2.47	93.0 ± 6.47	27.1 ± 2.47	93.6 ± 6.54	24.2 ± 2.11	127.7 ± 9.65	31.0 ± 3.27	147.6 ± 11.10	33.8 ± 2.07
	(10-129)	(0-117)	(10-243)	(0-109)	(16-230)	(0-105)	(11-300)	(0-128)	(40-384)	(0-70)

[b]beginning 5/1/98, [c]ending 12/31/02

Aerial Survey Data (1989-2002)

The linear regression on the CDFG counts (1982-2002) revealed a slight increase in the numbers counted in SFB (r^2=0.23, P<0.05) (Fig. 5). No significant trend was seen in the summed counts for the three haulout sites, CR, YBI and MNS (r^2=0.03, P>0.47) (Fig. 5).

A)



B)

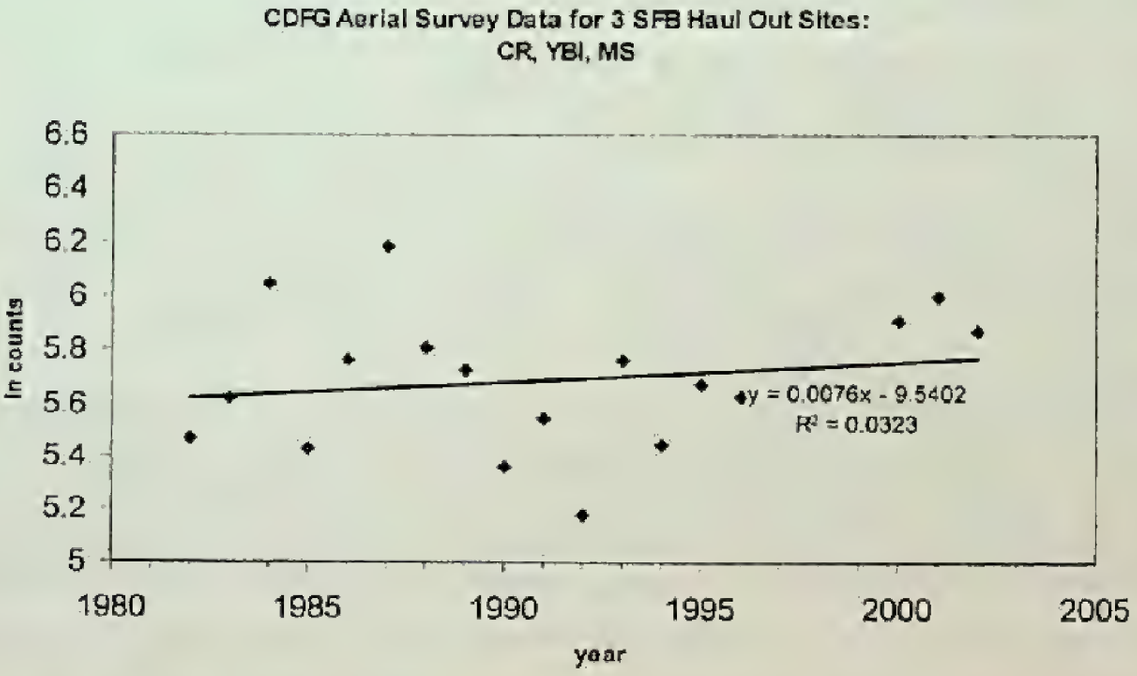


Figure 5: CDFG aerial survey data for SFB, 1982-2002. A) Counts from all SFB haul out sites combined; B) counts for three sites (currently the largest in SFB): CR, YBI, MS (sources: Fluharty 1999, Read and Sweetnam 2002; R.Read, CDFG, pers. comm. 2003)

DISCUSSION

Since passage of the Marine Mammal Protection Act (MMPA) in 1972 in the United States, several studies have documented the recovery of species, particularly of pinnipeds such as harbor seals (Allen et al. 1989, Harvey et al. 1990, Stewart et al. 1988, Sydeman and Allen 1999, Jeffries et al. 2003). Within a large urban estuary such as SFB, though, we document a mixed response and recovery of harbor seals that was likely due to a combination of factors including habitat alteration, disturbance, pollution, prey availability and/or survey techniques.

Our analyses revealed different trends in numbers of seals using the three primary SFB haulout sites across years and in seasonal use patterns for each site. We documented from the long-term (1970-2002) dataset, 1) no change in adult/subadult seal numbers at MNS during the pupping/molting seasons, 2) an increase in seal numbers at CR for all seasons, and 3) an increase at YBI during all seasons, followed by a leveling-off in recent years (1989-2002) during the fall/winter season. Generally, annual rates of change of <10% are considered small for harbor seal populations (Thompson et al. 1997), and no sites in SFB achieved that growth rate over the past three decades.

When looking at historical data in SFB, the growth rates at CR of 6.1% (pupping/molting seasons) and 5.3% (fall/winter seasons) reflect a small but steady increase in numbers of seals using this site. This site is tide and space limited, which may explain in part the low rate of increase. Corresponding with the increase in adult/subadult seal numbers at CR, the number of pups also increased over the years. Despite major retrofit construction of a bridge adjacent to the haulout site from 2001-2002, seal numbers at CR continued to increase during those years.

Numbers of seals at YBI increased sharply following the establishment of the site in the 1980's with an estimated growth rate of 9.0% since 1989. The increase of seals at YBI coincided with the abandonment of a site at Strawberry Spit (Allen[1] 1991) and initially with the winter herring spawn in SFB (Spencer[16] 1997). However, more recently, numbers also increased during the pupping/molting season (Galloway[19] 2000, Green et al.[15] 2002). Nevertheless, few pups occur at YBI compared to the other two sites in SFB. YBI is exposed to more human disturbance than the other two sites (Green et al.[15] 2002). Since 1999, though, the numbers have leveled off, suggesting density-dependency since space is limited at this location. In addition, in late 2002, a major disturbance event occurred, involving a 212-m dry dock grounding on YBI, that temporarily restricted seal access to the site.

In contrast with CR (the north bay pupping site), numbers of adult/subadult seals present during the pupping/molting season at MNS, the largest pupping site in SFB, have not changed significantly over the years since 1970. Higher seal numbers were recorded during the 2001-2002 pupping seasons, but whether these two years represent the beginnings of a population increase at MNS will require continued monitoring. MNS was one of the earliest haulout sites discovered in SFB (Bonnot 1928) and seals

[19]Galloway, M.J. 2000. Factors influencing scanning rates of harbor seals at Yerba Buena Island, California. M.A. Thesis, San Francisco State University, San Francisco, CA. 90 p.

have apparently been using the rookery for thousands of years (Fancher[2] 1979). MNS is a relatively undisturbed site located in a wildlife refuge and accessible at a wide range of tidal heights. The lack of increase of seals at MNS since passage of the Marine Mammal Protection Act, when contrasted with increases at many nearby coastal haulout sites at Point Reyes (Sydeman and Allen 1999) during the same time period, suggests that other factors may be influencing productivity at MNS.

Trends in seasonal differences between sites in SFB confuse these apparent trends. During the fall/winter season at MNS, for example, the growth rate was 3.4%, and the long-term growth rate at YBI was greater during the pupping/molting than during the fall/winter seasons. In addition, there was a time lag between the increase in use of YBI by adult/subadult seals during the pupping/molting season, and the start of the increase in pup numbers at this site, with increases in pup numbers only seen after 1998.

Fancher[2] (1979) noted in the 1970's that there was a strong seasonal influx of seals to MNS during the pupping/molting season and suggested that two groups of seals were using the site: a resident population of around 50-70 seasonal and a larger transient group that moved into the area to breed. Even taking into account the reduced amount of time harbor seals spend on the haulout site outside of the pupping/molting season (Allen Miller[7] 1988, Thompson et al. 1998), there appeared to be a large seasonal immigration of seals to the MNS area during the breeding season. Fancher[2] (1979) and Risebrough et al.[5] (1980) suggested that many of the seals present in SFB for the breeding season came from outside of SFB, and recent telemetry data support this conclusion (Torok[20] 1994, Green et al.[15] 2002). Green et al.[15] (2002) noted that 29% of seals tagged in SFB used coastal haulout sites over a one-year period. Other researchers (Brown and Mate 1983, Harkonen 1987, Jeffries et al. 2003) have observed similar trends where coastal seals move into estuaries for breeding. Consequently, examining trends in fall/winter when resident seals are present may be more relevant in determining population increases at MNS.

Harkonen (1987) suggested that because harbor seals primarily feed on benthic prey and do not fast during the breeding/molt seasons, the large seasonal influx of seals into an estuarine habitat results in a local depletion of prey resources. Consequently, many seals may leave an estuarine area after pupping/molting in search of more abundant prey, leaving behind a small number of seals that are supported year-round by local estuarine resources. Limited prey during the breeding/molt seasons would explain the smaller number of year-round resident seals at MNS and could explain the low rate of increase of SFB seals since passage of the MMPA. When prey abundance increased in SFB during the winter months as a result of herring spawning in the bay, seasonal increases in seal numbers were seen at the sites closest to herring spawning areas, CR and YBI. In addition, these sites also reveal an annual trend upwards over the past two decades. And too, the increase in harbor seal populations at coastal sites

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[20]Torok, M.L. 1994. Movements, daily activity patterns, dive behavior, and food habits of harbor seals (*Phoca vitulina richardsi*) in San Francisco Bay, California. M.S. Thesis California State University, Stanislaus and Moss Landing Marine Labs, Moss Landing, CA. 88 p.

(Allen et al. 1989, Sydeman and Allen 1999) would be reflected in the increased numbers of seals moving into SFB in response to localized prey increases.

To understand trends in numbers of harbor seals using the three haulout sites in SFB, changes in use at other haulout sites in SFB should be reviewed. Harbor seals have been reported to shift site use in response to prey movements, disturbance or other factors (Paulbitski 1975, Risebrough et al.[5] 1980, Brown and Mate 1983, Harkonen 1987, Allen[1] 1991, Greenstreet et al. 1993, Thompson et al. 2001). Consistent annual data for all sites in SFB do not exist; however, there are data on significant historical increases or decreases at some SFB sites. For example, Strawberry Spit, where around 100 seals annually hauled out in the 1970's, was abandoned in the early 1980's due to disturbance and a shift in prey resources (Fig. 1; Paulbitski 1975, Allen[1] 1991). Strawberry Spit itself was used only after seals were disturbed from a previous historical haulout site at Strawberry Point (Allen[1] 1991). Bartholomew and others documented the abandonment of a harbor seal haulout site at the foot of the San Francisco-Oakland Bay Bridge in the 1940's, possibly in response to increased disturbance from a nearby naval base (Fig. 1; Bartholomew 1949, Risebrough et al.[5] 1980).

More recently, new haulout sites have been documented in SFB over the past two decades. Seals began using Corte Madera Marsh in northern SFB in the 1980's (Fig. 1), coincident with the abandonment of Strawberry Spit and the restoration of marsh habitat (Allen[1] 1991). The SFB delta, in the northeastern-most reaches of SFB, was used by seals in the 1930's and 1940's, but by the 1970's, seals were only seen occasionally in the area (Paulbitski and Maguire 1972). In 1993, aerial surveys conducted by CDFG identified a new haulout site on Ryer Island, Suisun Bay (Fig. 1), where numbers have risen from 10 seals in 1993 to 53 in 2002 (R. Read, personal communication). These new haulout sites may be absorbing seals shifting away from areas that were abandoned, or may represent real increases in seal numbers in SFB. Recent data suggest that the current harbor seal population in SFB is somewhat higher than the CDFG estimate (Green et al.[15] 2002; Lowry and Caretta[21] 2002; the present study).

Understanding trends at individual sites in SFB is an easier task than defining a population trend for all of SFB. Strong environmental signals such as El Niño Southern Oscillation (ENSO) events are uniformly detected in harbor seal population numbers across all sites, both in SFB (as seen in this study during the 1998 ENSO event) and at coastal sites (Allen et al. 1989, Sydeman and Allen 1999). Other factors affecting SFB harbor seal population trends, such as disturbance or pollutants, may be more difficult to interpret. As Hanan[3] (1996) noted, recent increases in survey numbers could simply be the result of improved censusing techniques; however, recent data reflect the same overall trends seen in the historical data, and improved censusing alone may not explain the recent increases.

The CDFG annual surveys conducted during the molting season show a slight increase in the SFB population, but the annual counts were based on one aerial survey

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[21]Lowry, M.S. and Carretta, J. (2002) Pacific harbor seal, *Phoca vitulina richardii*, census in California during May-July 2002. NOAA Technical Memorandum NMFS-SWFSC-353. 77 p.

per year and, for SFB, were timed to fall at medium to low tides (Fluharty[11] 1999), which does not consistently coincide with the maximum numbers of seals present on all SFB haulout sites (Risebrough et al.[5] 1980, Kopec and Harvey[6] 1995, Green et al.[15] 2002). CDFG surveys, though, did not detect any increase at individual sites such as CR, MNS, and YBI (Fig. 5). This would suggest that if trend sites are to be used to monitor seal numbers in a given area, once-annual aerial surveys would not be sufficient to understand local population trends or seasonal patterns of use. CDFG surveys are not suitable for detecting population tends in areas such as SFB but are used only to detect trends in the state population (R. Read, personal communication). Consequently, regional managers must rely on local long-term monitoring data to guide management actions.

We recommend that in SFB, harbor seals should be surveyed annually during all four seasons at the three primary SFB haulout sites in order to accurately detect SFB population trends. Given that seals may shift haulout site location in response to disturbance and/or prey movements, data from trend sites should be used in conjunction with the annual California statewide aerial surveys, to aid in the interpretation of aerial survey data, and to document any new or lost seal haulout sites. Similarly, a complete understanding of seasonal and yearly use of certain significant haulout sites is important, providing an understanding of seal population trends that may not be available from once-yearly aerial surveys alone.

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EFFECT OF WATER TEMPERATURE ON NON-SPECIFIC IMMUNE FUNCTION AND CERATOMYXOSIS IN JUVENILE CHINOOK SALMON AND STEELHEAD FROM THE KLAMATH RIVER

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The potential immunosuppressive effect of elevated water temperature on resistance to *Ceratomyxa shasta* infection and disease (ceratomyxosis) was assessed in juvenile Klamath River chinook salmon, *Oncorhynchus tshawytscha*, and steelhead, *Oncorhynchus mykiss*. Mortality and clinical signs of ceratomyxosis were similar between juvenile salmon infected with *C. shasta* and then reared at water temperatures of 16° and 20°C. A 3-day (d) exposure in the upper Klamath River, during June 2002, resulted in a 100% prevalence of infection and mortality of ≥ 83% within 17-d post-exposure among salmon at both water temperatures. Measures of non-specific defense mechanisms (plasma complement and lysozyme activities, and anterior kidney cell phagocytosis) in uninfected, control fish held at 16° and 20°C were similar to each other and within normal ranges. In contrast, plasma lysozyme activity of *C. shasta* infected salmon was approximately 2X higher than control fish. Phagocytosis and plasma complement activity of infected and control salmon were similar until ceratomyxosis was quite advanced. Non-specific immune parameters were not affected in salmon held at 20°C. It appears the magnitude of parasite challenge has a stronger influence on disease severity than rearing temperature. Regardless of rearing temperature, salmon challenged with an intra-peritoneal injection of *C. shasta* trophozoites demonstrated a strong inflammatory defense against the parasites. Juvenile steelhead exposed for 3-d to Klamath River water and then reared at 14°, 18°, and 20°C did not develop ceratomyxosis. *Ceratomyxa shasta* was not detected in the exposed steelhead by either Polymerase Chain Reaction (PCR) assay or histology. Salmon exposed along with the steelhead experienced typical ceratomyxosis. Steelhead rearing or migrating in the Klamath River would appear to be at low risk for ceratomyxosis. When exposed to high *C. shasta* actinospore levels, the ecological outcome (death) for salmon will tend to be equal under moderate spring temperatures as well as higher summer temperatures.

INTRODUCTION

Ceratomyxa shasta is a histozoic myxosporean parasite of salmonid fishes that occurs in a number of watersheds in the Pacific Northwest and whose lifecycle includes

the polychaete, *Manayunkia speciosa,* as its alternate host (Hoffmaster et al. 1988, Bartholomew et al. 1997). Initial infection of the intestinal epithelium can progress into hemorrhagic enteritis and spread to other organs and into the peritoneum (Bartholomew et al. 1989). The disease is referred to as ceratomyxosis and is often fatal. *Ceratomyxa shasta* infection can occur from spring through autumn at water temperatures ≥ 7°C (Ching and Munday 1984, Hendrickson et al. 1989). Hendrickson et al. (1989) described the parasite's distribution in California to include the San Joaquin, Sacramento, Pit, and Klamath River systems. High mortality due to ceratomyxosis has been observed in juvenile salmon out-migrants in the Klamath River basin (Foott et al. [1]1999 and [2]2002). Elevated water temperatures, commonly in excess of 20°C in the Klamath River during the late spring and summer, act to accelerate the disease process (Udey et al. 1975). There is a large variation in susceptibility to ceratomyxosis among salmonid fishes with resistant strains being found primarily in enzootic drainages (Zinn et al. 1977, Buchanan et al. 1983, Iberra et al. 1991). A high degree of resistance was documented in Klamath River juvenile chinook salmon exposed to Pit River water with the infectious stages of the parasite (Foott et al. [1]1999). In this paper we characterize the effects of elevated water temperature on both the resistance to ceratomyxosis and several non-specific immune functions of juvenile chinook salmon and steelhead following exposure to the infectious stages of *C. shasta.*

METHODS

On 21 June 2002, four hundred juvenile fall-run chinook salmon, *Oncorhynchus tshawytscha*, from the California Department of Fish and Game's Iron Gate Hatchery (Hornbrook, CA) were placed in the Klamath River upstream of Beaver Creek (rkm 262). Live box exposures in May had shown this site to be highly infectious for *C. shasta.* The salmon were held in river water (average 20.5°C) for 3 days (d) within 0.03 m^3 live boxes and then transported to the California-Nevada Fish Health Center wet laboratory for extended rearing. These salmon are referred to as exposed fish. Approximately 450 salmon (referred to as exposure controls) were also held for 3-d within live boxes at the hatchery and transported to the wet laboratory at the same time. In order to reduce *Flavobacterium columnare* infections, both groups received a 10 min, prophylactic bath of 1 mg/L furanase (Aquarium Products, Glen Burnie MD) upon arrival at the laboratory. The groups were dispersed as 24 fish replicates into 40 liter (L) aquaria supplied with aeration and 8 L/min flow of either 16° or 20°C water. Each 1860-L re-circulation system had 20 aquaria, a 760-L temperature conditioning tank with chillers

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[1] Foott, J.S., J.D. Williamson, and K.C. True. 1999. Health, physiology, and migration characteristics of Iron Gate Hatchery Chinook, 1995 Releases. U.S. Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson CA.

[2] Foott, J.S., T. Martinez, R Harmon, K. True, B. McCasland, C. Glase, and R Engle. 2002. Juvenile Chinook Health Monitoring in the Trinity River, Klamath River and Estuary, June – August 2001. U.S. Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson CA.

and heaters, a 300-L effluent sump with chillers and heaters, and pumps. Freshwater inflow was set at 38 L/min. A commercial salmon diet was offered twice a day. Water temperature was monitored every 2 hrs with Onset™ Stowaway temperature loggers. Once a day, dissolved oxygen was measured with a YSI 95 meter and chemical analysis kits (Hach Company, Loveland CO) were used for both pH and ammonia assays. The laboratory effluent was treated with 5 mg/L chlorine for 50 min and then largely removed by filtration through activated charcoal filters prior to discharged into a 1.3-ha abatement pond.

The effect of water temperature on early infection responses was examined in salmon injected with *C. shasta*. At 10-d post-exposure (dpe), intestinal contents from 10 clinically ill fish were pooled and added to 5 mL cold Hanks Buffered Salt Solution (HBSS) with 1600 units / mL Penicillin G – 1600 µg/L Streptomycin sulfate. A hemocytometer count of the suspension showed 285 trophozoites/µL as well as host cells and debris. Thirty uninfected control fish were given a 0.1 mL intraperitoneal injection of the suspension and divided between 16° and 20°C aquaria (fish identified as IPE). Injection controls (IPC) received 0.1 mL of HBSS.

On 19 July 2002, two hundred juvenile steelhead, *Oncorhynchus mykiss*, were exposed for 3-d in the Klamath River (average 23.2°C) at the same site used for the chinook exposures. One live box of 30 chinook salmon was also exposed at the site and the fish later reared at 20°C. Only 59 steelhead were recovered from the live boxes for the experiment. We assume the other steelhead escaped from a tear in the live box netting. Exposure control steelhead (C) were held at the hatchery and transported to the wet laboratory at the same time as the exposed group. These fish were handled in a similar manner as the salmon in the June experiment except only 4 - 8 fish per group were placed into replicate aquaria maintained at 14°, 18°, and 20°C. No injection challenges were performed on the steelhead. Both the chinook salmon and steelhead are progeny of Klamath River stock adults that returned to Iron Gate Hatchery.

Twenty salmon from each group were sampled at 10 and 17 dpe. The sample size for the infected exposed salmon declined to 8 fish by 17 dpe due to mortality. Ten injected salmon from each group were sampled at 7 dpi. Steelhead were sampled at 3, 10, 17, and 24 dpe. Sample size ranged from 3 – 6 fish per group for each sample period. Fish were euthanized by an overdose of tricaine methanesulfonate, measured for both fork length and weight, and examined for external abnormalities such as swollen abdomen, pale gill, or hemorrhagic vent. The caudal peduncle was then cut and blood collected into heprinized microhematocrit tubes. The tubes were centrifuged at 10,000 x g for 5 min., a hematocrit measurement taken, and the plasma stored frozen on dry ice in two aliquouts (15 µL for the complement assay, and 12 – 20 µL for lysozyme and total protein determinations). Plasma samples were stored at -70°C until assayed. In the control salmon, gill tissue was collected for Na-K-ATPase assays. Upon dissection, internal abnormalities such as intestinal hemorrhage or swollen kidney were noted for each fish. Intestinal tract (including intestinal caeca) and kidney was rapidly removed from the fish after blood sampling and placed in Davidson's fixative, processed by routine procedures and were stained with hematoxylin and eosin (Humason 1979). All tissues for a given fish were placed on one slide and identified by a unique code number.

Each slide was examined at both low (40X) and high (400X) magnification without knowledge of the sample group. Prior to histological sampling, a 1 - 2 mm section of lower intestine was removed by DNA-free tools (disposable sticks and scalpels that were bleached and 2X washed in distilled water) for storage at -70°C as a PCR archive sample. Several intestinal samples from exposed steelhead, that did not show clinical or histological signs of *C. shasta* infection, were tested for specific parasite DNA by the Polymerase Chain Reaction (PCR) method of Bartholomew (2001).

The lysozyme activity of plasma (mOD / min.) was determined from 5 µL samples assayed by a turbidimetric method using a pH 6.4 buffer (Ellis 1990). Alternative complement activity, in 15 µL plasma samples, was determined by the degree of rabbit erythrocyte hemolysis (Alcorn et al. 2002). Low sample volume (15 µL) limited the assay to a single dilution that precluded a calculation of the amount of plasma that would lyse 50 % of the rabbit erythrocytes (ACH_{50} value). Hemolytic activity of the samples was reported as a percent (OD_{540} of sample / OD_{540} of 100% hemolysis control). Total plasma protein concentration (g / dL) was assayed by the biuret method (Sigma Chemical Co., kit No. 541). Due to limited sample volume, only plasma protein was tested in steelhead. Anterior kidney cell phagocytosis was assessed by the bactericidal method described by Secombes (1990). Briefly, a suspension of 2 x 10^6 anterior kidney cells were mixed with 10^7 *Yersinia ruckeri* and allow to react for 3 hr at 15°C. Positive controls were without kidney cells. The concentration of surviving target bacteria was estimated by spectrophotometry using the mitochondrial dye MTT (3(4,5-dimeththiazoly-2-yl 2,5 diphenyltetrazolium bromide)) and the percent kill calculated as $OD_{\text{kidney cells}} / OD_{\text{positive control}}$. Gill Sodium-PotassiumAdenosine Triphosphatase activity (ATPase = µmoles ADP / mg protein / hr) was assayed by the method of McCormick and Bern (1989).

RESULTS

Rearing temperatures for both salmon and steelhead after live-box exposures to *C. shasta* were maintained within 0.5°C of the target. Other water quality parameters were maintained within normal ranges for salmonids (dissolved oxygen ranged from 5.7 – 9.6 mg /L, pH from 7.5 – 8.0, and unionized ammonia was below 0.05 mg / L). Rearing temperature did not alter the mortality pattern among the exposed salmon groups. The first mortality, associated with ceratomyxosis, occurred 7 dpe and mortality in both groups peaked at 13 – 14 dpe. (Fig.1). Mean day to death was 12.858 days for the 16°C fish and 13.884 days for the 20°C exposed fish. This difference was statistically significant (Kaplan-Meir Survival analysis log rank score of 54.437, 1 df, $P<0.01$). The experiment was concluded at 17 dpe as only 8 live salmon remained in each exposed group. Excluding sampled fish, cumulative mortality for the 16°C exposed group was 82.4% (155 dead of 188 total) and 89.9% (212 dead of 236 total) for the 20°C group. No mortality occurred in the control groups. Ten fish among the exposed groups were observed at 7 dpe to have clinical signs of *Flavobacterium columnare* infection (columnaris) and were removed from the experiment. Both control and exposed groups were then treated with a second 30 min static bath of 1% furanase. No additional observations of columnaris were made for the remainder of the experiment. An

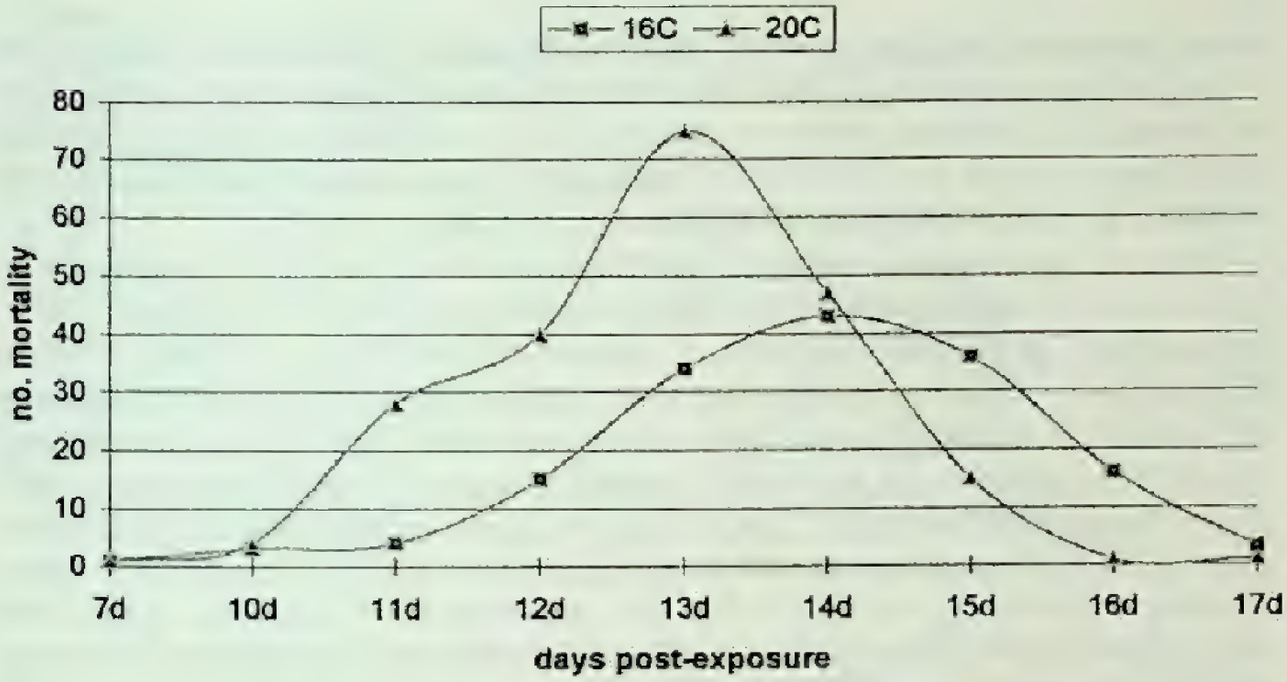

Figure 1. Ceratomyxosis mortality curve for juvenile chinook salmon exposed for 3 d to the Klamath River and reared at 16° and 20°C. Cumulative mortality for fish held at 16° and 20°C was 83% and 90%, respectively.

Ichthyophthirius multifiliis infestation was detected in fish held at both water temperatures on the final 17 dpe sample. In particular, both exposed and control salmon in the 20°C system had the characteristic white spot and displayed "flashing" behavior.

Mean fork lengths of the chinook ranged between 95 - 103 mm with mean weights ranging between 9.0 – 11.3 g . Anemia, as indicated by hematocrits below 20%, was observed in 50% of the exposed fish in both temperature groups at 17 dpe. Mean hematocrit values of the control salmon ranged from 33 – 35%. Multifocal hemorrhages were observed within the intestinal tract of all exposed fish. In histological sections of the intestinal tract, *C. shasta* trophozoites were seen primarily in the small intestine and intestinal ceca of all exposed fish. Initial infections were consistently observed in the small intestine while lower intestine involvement tended to occur at 13 dpe. The infection was associated with severe hyperplasia of mononuclear cells within the lamina propria, hemorrhage, and erosion of the intestinal epithelium. The intestinal lumen was often filled with necrotic host cells and trophozoites. By 17 dpe, trophozoites had often penetrated the intestinal tract and the infection had spread systemically in the fish. At this point, trophozoites were observed within inflammatory foci in the kidney, liver, and peripancreatic region.

All injected salmon were sampled at 7 days post-injection (dpi). No mortality occurred in either parasite or HBSS injected salmon during this period. No parasites were observed in kidney or intestinal tract sections from the HBSS – injected control fish. In contrast to the Klamath River exposed fish, relatively few *C. shasta* trophozoites were observed in tissue sections from 4 of 9 IPE-16°C (44%) and 5 of 10 IPE-20°C (50%) salmon. The parasites were associated with an intense inflammatory response on the

serosal surface of the intestinal tract, and in blood sinuses of the kidney and liver. No *C. shasta* trophozoites were observed within the intestinal tract of the injected fish. Necrotic *C. shasta* trophozoites were observed within inflammatory cell foci of several IPE salmon. Two of 10 IPE-20°C fish appeared to have advanced ceratomyxosis as indicated by high trophozoite numbers and peritonitis.

Another myxosporean parasite, commonly observed in kidney sections from chinook salmon collected throughout the Klamath basin (Foott et al. [1]1999 and [2]2002), was only seen in the kidney sections of exposed salmon (Fig. 2). The myxosporean differed from *C. shasta* in its light eosinophic staining characteristics of the cytoplasm and nuclei. In hematoxylin and eosin stained sections, *C.shasta* trophozoites have basophilic cytoplasm and nuclei that contain a large eosinophilic karyosome with distinct peripheral chromatin. The kidney myxosporean was most prevalent in the glomeruli and often associated with glomerulonephritis in the 17 dpe samples. Gross swelling of the kidney was observed in 33% of the infected 17 dpe fish. No parasites were observed in the kidney or intestinal tracts of either exposure or injection control salmon.

Plasma protein concentrations were significantly ($F = 31.95$, $df = 3$, $P < 0.001$) reduced in exposed salmon compared to the controls and by 17 dpe were less than 1.0 g /d L in the exposed fish (Fig. 3). There was no significant difference in the plasma protein levels

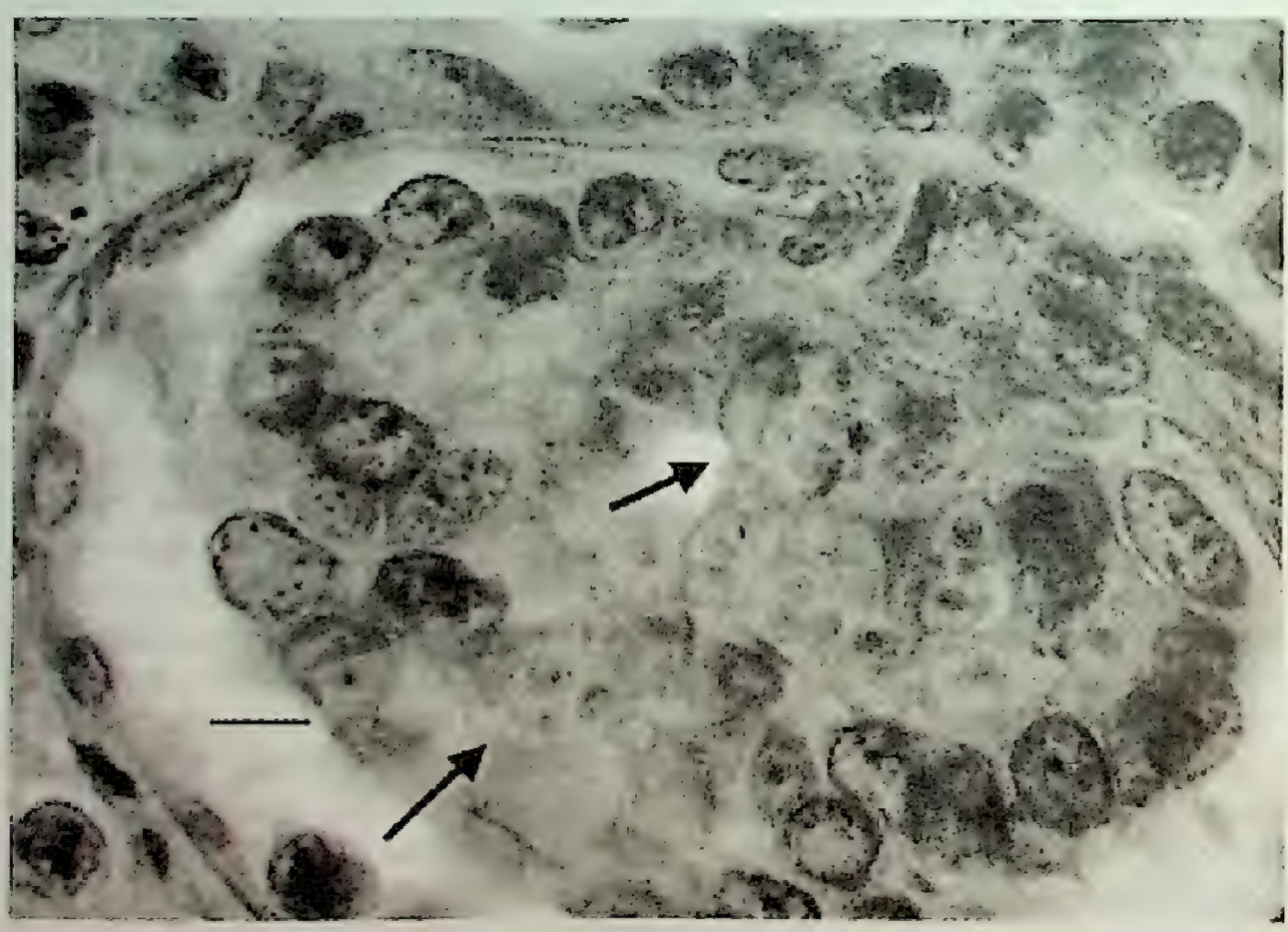

Figure 2. Myxosporean (presumptive *Parvicapsula minibicornis*) in section of kidney glomerulus from a chinook salmon. Parasite denoted by arrows, bar = 5 μm.

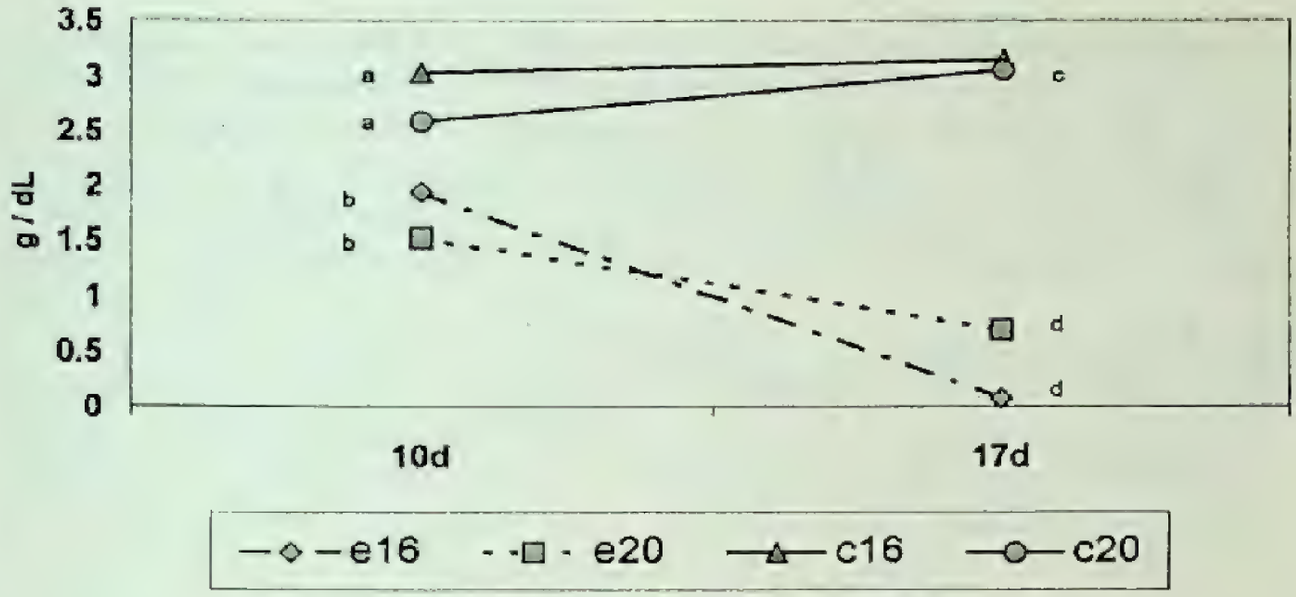

Figure 3. Mean plasma protein concentration (g/dL) of exposed (e) and control (c) salmon held at 16° and 20°C. Fish were sampled at 10 and 17 d post-exposure. . Letters indicate statistical difference at specific sample time (10 dpe, F = 31.95, df = 3, P<0.001 and 17 dpe, H = 31.03, df = 3, P<0.001).

between *C. shasta*- injected and HBSS-IP controls. The mean protein values for all injected groups were above 2.1 g/dL. Despite the low plasma protein levels of *C. shasta*-infected exposure salmon, both plasma lysozyme and complement activities tended to be elevated compared to the controls. Except for the 20°C control salmon at 17 dpe, lysozyme activity (mOD / min) was approximately 2X greater in exposed fish compared to the controls (Fig. 4). Salmon challenged by injection of the parasite also had elevated lysozyme activity values regardless of rearing temperature. Mean activities (± SEM) were 9.7 (±0.6), 5.6 (±0.60), 11.1 (± 1.4), and 9.7 (± 1.1) for the IPE16°, IPC16°, IPE20°, and IPC20° groups, respectively. Despite a 1.6 to 1.7X lower plasma protein concentration, complement hemolytic activity (% hemolysis) was similar among both exposed and control salmon from both temperatures at 10 dpe (Fig. 5). The mean percent hemolysis declined over time in all groups. By 17 dpe, exposed fish had lower values than control salmon held at the same temperature. There was no clear correlation between plasma protein concentration and percent hemolysis ($r^2 = 0.07$ and 0.21 for 10 and 17 dpe groups, respectively). Mean hemolytic activity (± SEM) of the 16° and 20°C parasite-injected salmon was 62 % (± 4) and 59%(± 7)) and was not significantly different than the 56 % (± 4) and 40% (± 7) of the 16° and 20° HBSS controls, respectively (F = 2.75, df = 3, P=0.062).

Phagocytic activity of anterior kidney cells, as measured by the MTT bactericidal kill ratio, was similar between exposed and control salmon at the same rearing temperature. Both 20° C groups had higher kill values than the 16° C salmon. At 10 dpe, the mean percent kill (± SEM) of the 16° C exposed and control fish were 66 % (±6) and 72 % (± 4), respectively. In comparison, the 20° C exposed fish had a mean percent kill of 98% (±0.2) and the controls 99% (±0.2). At 17 dpe all groups had ≥ 98 % kill values.

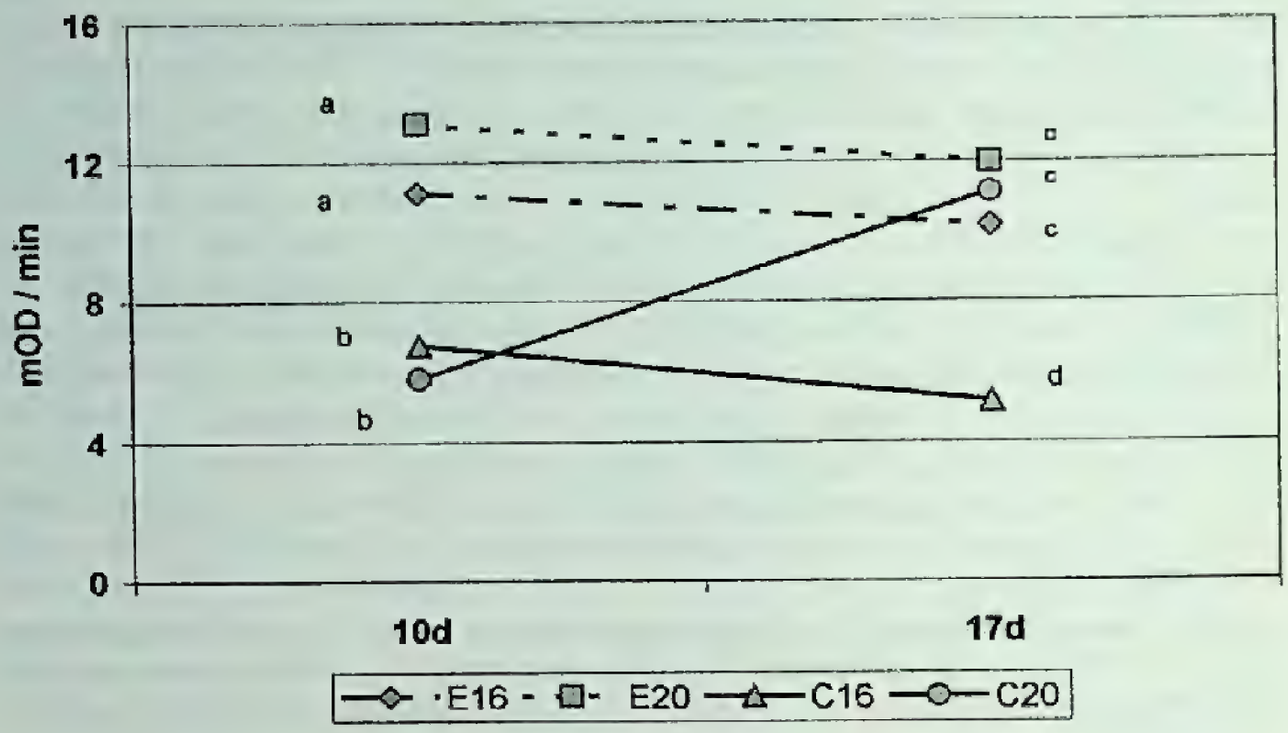

Figure 4. Mean plasma lysozyme activity (mOD/ min) of exposed (e) and control (c) salmon held at 16° and 20°C. Fish were sampled at 10 and 17 dpe. Letters indicate statistical difference at specific sample time (10 dpe, F = 34.71, df = 3, P<0.001 and 17 dpe, F = 10.41, df = 3, P<0.001).

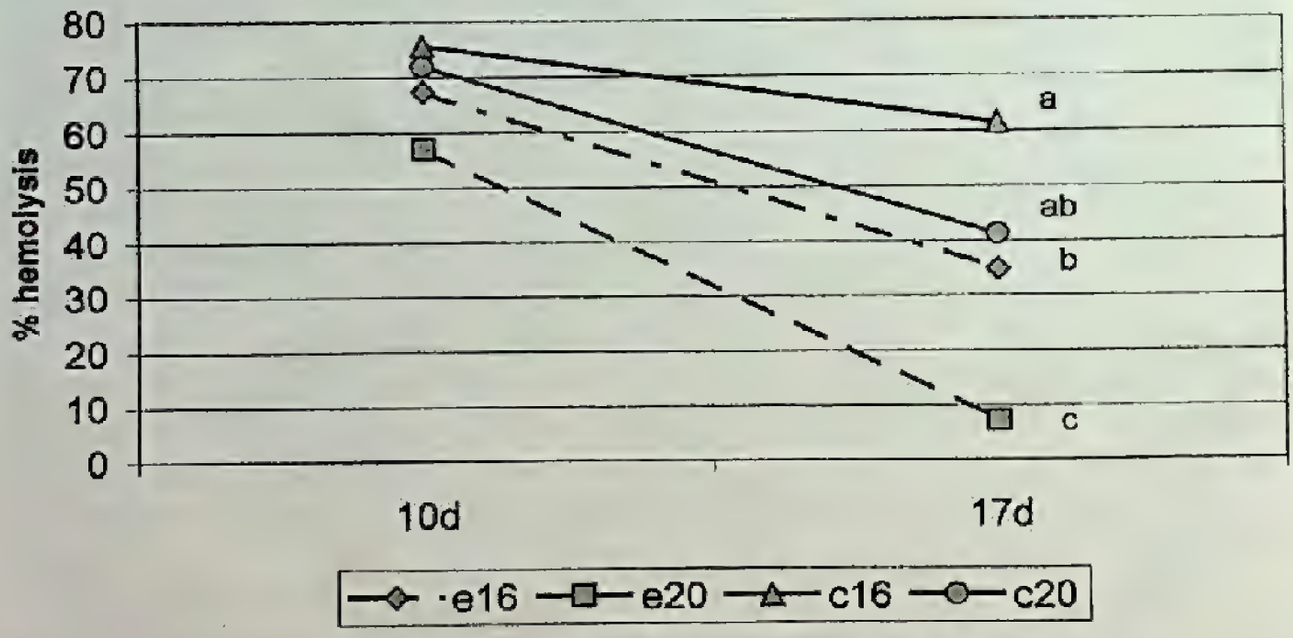

Figure 5. Hemolytic activity of plasma from control (C) and exposed (E) salmon held at 16° and 20°C. Fish were sampled at 10 and 17 dpe. Alternative complement action against rabbit erythrocytes recorded as mean percent hemolysis. Letters indicate statistical difference at 17 dpe (F = 10.49, df = 3, P<0.001).

The ratio of *Y. ruckeri* bacteria to phagocytes appeared to be too low for the 17 dpe assay. Gill ATPase activity (μm ADP/mg protein/hr) declined over time in both control groups with the lowest values occurring in the 20°C salmon. At 10 dpe, mean activity (± SEM) was 7.5 (± 0.7) in the 16°C control salmon and 6.3 (± 0.3) in the 20°C control salmon. Activity declined to 5.8 (± 0.7) in the 16°C salmon and 4.3 (± 0.6) for the 20°C group by 17 dpe. This decline was statistically significant between the 20°C sample groups ($t = 3.027$, $P = 0.009$).

No mortality or clinical signs of infection occurred in either exposed or control steelhead groups over the 24-d experiment. Mean fork length tended to increase over the experiment in all groups and ranged from 52 – 62 mm. Average group weight ranged from 1.5 – 3.1 g. Hematocrits were above 30% and plasma protein concentration tended to increase over time in all groups. Mean plasma protein concentrations ranged from 2.4 – 3.8 g/dL. There were no significant differences ($F = 1.48$, $df = 5$, $P = 0.249$) in plasma protein between temperature groups at any given sample date. No *C. shasta* trophozoites or myxosporean kidney parasites were observed in histological sections from 47 exposed and 21 control steelhead sampled throughout the experiment. Samples of lower intestine from 3 steelhead collected immediately after the 3 d exposure, two steelhead sampled at 10 dpe, and one steelhead collected at 24 dpe were negative by PCR assay. Both *C. shasta* and the kidney myxosporean were seen in histological sections of chinook exposed along with the steelhead and sampled at 6 and 13 dpe. Unlike salmon exposed in June and sampled after 10 dpe, only limited numbers of *C. shasta* trophozites were observed in the epithelium of the small and large intestine from 3 of 6 salmon exposed in July and sampled at 6 dpe. These early infections were associated with localized inflammation but no necrotic changes were observed in the intestine. All three salmon sampled at 13 dpe had severe ceratomyxosis and two were also infected with the kidney myxosporean.

DISCUSSION

The high incidence of ceratomyxosis in chinook salmon smolts collected from the Klamath River and estuary indicate that this parasitic disease is a major mortality factor for both hatchery and natural populations. Over 40% of chinook smolts sampled from a lower Klamath River screw trap (rkm 81) have been diagnosed with severe ceratomyxosis (Foott et al. [1] 1999 and [2] 2002). The incidence of *C. shasta* infection has ranged from 29 –43 % in juvenile chinook collected in the Klamath estuary (Foott et al. 2002 [2], Nichols et al. 2003[3]). The Klamath River would appear to have all the elements necessary for the parasite's lifecycle: specific habitat(s) for the alternate polychaete host *Manayunkia speciosa*, a temperature profile that facilitates infection and accelerates the disease process, and the continual re-infection of the polychaetes from spores shed by infected salmonids (particularly adults). While Klamath River salmonids face the greatest threat, there is evidence Trinity River chinook smolts become infected and diseased after entering the Klamath on their out-migration. In the summer of 2002, nineteen percent of marked Trinity River Hatchery chinook salmon smolts collected in the estuary were

infected with *C. shasta* (Nichols and Foott [3] 2003). It is unlikely that many smolts with detectable infection survive the disease as ceratomyxosis has been shown to persist in juvenile salmon after their saltwater entry (Margolis and Evelyn 1975).

Kidneys of the exposed salmon contained a second myxosporean species that induced glomerulonephritis. This is a common condition for juvenile chinook salmon migrants in the Klamath River basin with an incidence of infection ranging from 45 – 84% (Williamson and Foott [4] 1998, Foott et al. [1] 1999 and [2] 2002, Nichols et al. [3] 2003). Affected fish often have swollen, pale kidneys. Impaired renal function may reduce the ability of infected smolts to adapt to higher salinities. This myxosporean was recently identified, as *Parvicapsula minibicornis,* in both PCR assay and in-situ hyrbidization of kidney tissue from Klamath River juvenile chinook (S. Jones, Pacific Biological Station, Nanaimo British Columbia, Canada, 2003,personal communication,). St-Hilaire et al. (2002) reported that *P. minibicornis* infection of Frazer River sockeye salmon was associated with high pre-spawn mortality. A similar myxosporean has also been described in kidneys of adult Rogue River (Oregon) chinook salmon diagnosed with glomerulonephritis (Meyer and McPherson 1985).

Despite being held at 20°C, chinook exposed along with the steelhead in late July were slower to develop clinical ceratomyxosis than salmon exposed in June. The mean daily temperature during the June exposure was 20.5°C while the July exposures was 23.2°C. It is possible that the concentration of infectious stages of *C. shasta* was lower in July than June due to elevated temperature effects on the parasite or its polychaete host. Markiw (1992) reported the actinospores of *Myxobolus cerebralis* became non-viable within 3d at 19 - 20°C compare to 7d at 7 C. Ratliff (1983) reported that *C.shasta* infections occurred in sentinel trout held below the thermocline in Lake Simtustus (Oregon) but not in cohorts held in the warm surface waters (≥ 18°C). Ratliff (1983) postulated that the infective stage was associated with the bottom and could not penetrate the thermocline. Another explanation is that high water temperatures limit the infectivity of the actinospore. If high summer water temperatures limit infectivity, seasonality of infection may be somewhat bi-modal in the Klamath system with peaks in the early spring and fall.

Suppression of non-specific immune defenses did not appear to be responsible for the poor resistance observed in the exposed chinook salmon. Control salmon reared for 17d at 20°C had similar non-specific immune defenses as cohorts held at 16°C. Additionally, the non-specific defenses of infected fish were either elevated or at levels similar to the controls until they were near death. When sampled at 10 dpe, the infected salmon demonstrated elevated lysozyme activities as well as similar phagocyte killing ability and complement activities as the controls. While complement activity did decline at 17 dpe, the infected fish had extremely low plasma protein concentrations.

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[3]Nichols, K., D. Therry, and J.S. Foott. 2003. Trinity River Fall Chinook Smolt Health following passage through the lower Klamath River, June – August 2002. U.S. Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson, CA.

[4]Williamson, J.D. and J.S. Foott. 1998. Diagnostic evaluation of moribund juvenile salmonids in the Trinity and Klamath Rivers (June-September 1998). U.S. Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson, CA.

Despite their moribund condition, phagocyte killing ability and lysozyme activity remained high in these infected 17 dpe salmon. Saeij et al. (2003) report that parasitic infection in carp resulted in up-regulation of mRNAs for acute phase proteins such as complement factor 3 , serum amyloid A, and alpha-2-macroglobulin. Plasma lysozyme activity appears to be a good indicator of parasitic infection as both *C. shasta*-infected fish and control salmon with *Ichthyophthirius multifiliis* infestations had elevated values. We have observed similar high lysozyme activity values in juvenile chinook salmon from the Merced River infected with the kidney parasite *Tetracapsuloides byrosalmonae* (the cause of proliferative kidney disease). Lysozyme is produced by both macrophages and neutrophils in fish, and increased serum activity has been associated with activation of these phagocytes by infection (Paulsen et al. 2001, Munoz et al. 2000, Balfry et al. 1997). When lysozyme values are converted to activity units (1 unit = mOD/min/mL of plasma), the mean activity measured in the control 16° and 20°C chinook, 1348 and 1160 units respectively, were similar to 1033 and 1400 units reported for healthy Atlantic salmon and rainbow trout serum, respectively (Lygren et al. 1989, Lie et al. 1999). The percent kill of *Yersinia ruckeri* by anterior kidney cells of both exposed and control salmon sampled at 10 dpe (66 – 99%) was higher than the 40 % reported by Siegel and Congleton (1997) for chinook salmon macrophages and *Aeromonas salmonicida* under similar assay conditions (10^6 cells and 10^5 bacteria). Shoemaker et al. (1997) observed 85.6 to 95.2 % kill of *Edwardsiella ictaluri* by channel catfish macrophages assayed under similar conditions as our study. While assay conditions such as different bacteria, cell concentrations, and reaction times limit comparison with other reports, both the exposed and control chinook salmon phagocytes showed a strong bactericidal response at 10 dpe.

The exact mechanism(s) for ceratomyxosis resistance has not been described in the literature. Bartholomew et al. (1989) reported that they could not detect a specific immune response (immunoglobulin) in salmonids naturally infected with *C. shasta*. The authors speculate that exclusion of the parasite from the intestine is the primary defense mechanism but that super-infection can overwhelm this defense. Ibarra et al. (1992) discuss the effects of high challenge dosage on resistance. They reported that progeny from crosses of resistant and susceptible trout parents showed intermediate levels of resistance to ceratomyxosis when challenged by a 7-d exposure to waters containing *C. shasta* infectious stages. These same F1 hybrids experienced significantly higher mortality due to ceratomyxosis when challenged by continuous exposure (>120 d). Similarly, intraperitoneal injection of large numbers of trophozoites and pre-sporogonic stages into resistant strains of trout resulted in lethal ceratomyxosis and was dose-dependent (Ibarra et al. 1991). Such a challenge would bypass the epithelial defenses of the intestinal tract. In our study, a number of salmon injected with a crude parasite preparation appeared to be successfully containing the infection with inflammatory cells. It is likely that the single challenge event was below a threshold level required for superinfection and subsequent disease progression. Juvenile chinook salmon and steelhead from Iron Gate Hatchery demonstrated resistance to *C. shasta* in a 1995 and 1996 exposure study at the California Department of Fish and Game's Crystal Lake hatchery. No mortality or histological signs of infection occurred in fish exposed to

infectious Pit River water for 20 - 85 d (Foott et al.[1]1999). In contrast, susceptible rainbow trout (Shasta strain) exposed with the Iron Gate fish suffered complete mortality due to ceratomyxosis. The results of this limited study were not consistent with observations of ceratomyxosis in hatchery salmon captured in the Klamath River and estuary. Water temperature during the 1995 and 1996 exposures were below 17°C and the challenge titer of the water was unknown. It is possible that the concentration of infectious actinospores in the Pit River water was below a threshold level to induce infection in the Iron Gate chinook during these exposures.

Klamath River steelhead were completely resistant to ceratomyxosis when challenged and later reared at water temperatures up to 20°C. This salmonid population would appear to be at low risk for ceratomyxosis when rearing or migrating in the Klamath River. The impact of ceratomyxosis on coho salmon in the Klamath River is not known. One moribund coho salmon juvenile was recovered at the mouth of Pecwan creek (rkm 40) in July 2001 and demonstrated *C. shasta* trophozoites in histological sections (Foott et al. [2] 2002). Udey et al. (1975) reported that juvenile coho salmon demonstrated a temperature-related ability to defend themselves against lethal ceratomyxosis following a 3-d exposure to infectious water. The authors report that a 201-d cumulative mortality was 84% at 20.5°C and only 22% at 15.0°C. The geometric mean time to death for the infected coho was 19-d at 20.5°C and 45-d at 15.0°C. In our study, the magnitude and rate of mortality was not markedly different between chinook salmon held at 16° and 20°C. It appears the magnitude of parasite challenge has a stronger influence on disease severity than rearing temperature. While the mortality rate of infected salmon at 20°C was slightly greater than the 16°C group, there was no appreciable difference in the level of severe ceratomyxosis experienced by both groups. Thus ecological outcome (death) for salmon challenged at such high *C. shasta* actinospore levels would be equally lethal under moderate spring temperatures as well as higher summer temperatures.

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PERSISTENCE OF IDENTIFIABLE REMAINS OF WHITE STURGEON JUVENILES IN DIGESTIVE TRACTS OF NORTHERN PIKEMINNOW

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Juvenile white sturgeon, *Acipenser transmontanus*, have not been commonly identified as prey items in digestive tracts of fishes collected in the wild. In particular, the diet of northern pikeminnow, *Ptychocheilus oregonensis*, an abundant Pacific Northwest freshwater predator which has been widely studied, has not included juvenile white sturgeon. To aid in interpreting these results and help in planning future feeding studies, we determined the persistence of identifiable remains of white sturgeon juveniles in this predator's digestive tract. Northern pikeminnow (mean total length = 476 mm), were force-fed meals of 2 or 3 juvenile white sturgeon (mean total length = 91 mm). After digestive periods of 4, 8, 16, 24, 28, and 32 h at a water temperature of about 17 °C, fish were sacrificed, digestive tracts removed, and contents examined. Our results indicate that juvenile white sturgeon would be readily discernable in digestive tracts of northern pikeminnow at least a day after feeding, with scutes remaining undigested and identifiable for 28 h.

INTRODUCTION

Adult white sturgeon, *Acipenser transmontanus*, occur throughout much of the Pacific Northwest, but in many locations their population sizes are diminishing. This is partly because during some years little or no recruitment of young-of-the-year white sturgeon is observed despite evidence of spawning (Parsley et al. 2002). Predation might be one cause of young white sturgeon mortality, and predators such as northern pikeminnow, *Ptychocheilus oregonensis*, have been observed to eat white sturgeon larvae and juveniles in the laboratory (D. Gadomski, personal observation). However, in studies that have described diets of 8152 northern pikeminnow, 6291 smallmouth bass, *Micropterus dolomieu*, 1182 walleye, *Sander vitreus*, and 655 channel catfish, *Ictalurus punctatus*, in the Columbia River Basin (Poe et al. 1991, Zimmerman 1999), only the ingestion of one white sturgeon juvenile has been reported (Parsley et al. 2002).

It is unknown if these predators are simply not eating white sturgeon juveniles or if other factors are causing these results. It is possible that because white sturgeon have cartilaginous endoskeletons they are more quickly digested than fishes with bony skeletons, and thus not easily detected in fish digestive tracts. Our objective was to determine the persistence of identifiable remains of white sturgeon juveniles in a

predator's digestive tract. We used northern pikeminnow as the predator in laboratory trials because this native species is common in much of the range of white sturgeon and has been the focus of many feeding studies (Thompson 1959, Poe et al. 1991, Petersen et al. 1994, Ward et al. 1995). Also, northern pikeminnow are known to feed on benthic organisms, particularly in the absence of juvenile salmonids (Poe et al. 1991, Zimmerman 1999), and thus could potentially prey on young white sturgeon in the wild. Furthermore, work has been conducted on digestion and evacuation rates of northern pikeminnow that have eaten juvenile salmon and other fishes (Steigenberger and Larkin 1974, Beyer et al. 1988), which would allow us to compare our results to these studies.

METHODS

Northern pikeminnow (mean total length, TL = 476 mm, standard error, SE = 15 mm, n = 14) were collected by boat electroshocking and angling from the Columbia River about 5 months before experiments were initiated. They were held in the laboratory in indoor 2.4-m diameter circular tanks (0.9-m depth) with heated flow-though well water at 17 ± 0.5 °C. Overhead lighting mimicked a natural photoperiod with light levels gradually increasing at dawn and decreasing at dusk. Prior to being used in trials, northern pikeminnow were fed a maintenance diet of live juvenile white sturgeon (mean TLs ranging from 37 to 106 mm, SE = 1 and 2, respectively, n = 20 for both groups), and juvenile chinook salmon, *Oncorhynchus tshawytsch*, and coho salmon, *O. kisutch* (mean TLs ranging from 68 to 111 mm, SE = 1 and 2, respectively, n = 10 for both groups), all obtained from local hatcheries. White sturgeon (mean TL = 91 mm, SE = 2 mm; mean weight = 4 g, SE = 0.03 g; n = 36) used in experiments were held in indoor 1.2-m diameter tanks (0.7-m depth) with flow-through well water at 17 ± 0.5 °C and fed commercial semi-moist salmon diets to excess.

A week before trials were conducted, northern pikeminnow were transferred to 1.2-m diameter tanks with flow-through well water at 17 ± 0.5 °C, the experimental temperature. Food was withheld from predators for about 4 days before an experiment. To initiate a trial, we selected white sturgeon to be used as prey and weighed and measured them. A northern pikeminnow was then netted and placed in a rounded trough lined with polyurethane foam and restrained with a foam overlay. No anesthetic was used. A 20-mm outer-diameter, 15-mm inner-diameter polyvinyl chloride rigid pipe with beveled and sanded edges was inserted through and past the pharyngeal teeth to the foregut. White sturgeon to be used as prey were killed with a blow to the head and pushed head-first one at a time through the pipe into the gut of the northern pikeminnow using a plastic rod. After a meal was inserted, the pipe and rod were removed and the northern pikeminnow was placed in a 1.2-m diameter tank.

We force fed a predator 2 or 3 white sturgeon, although regurgitation caused some meal sizes to be smaller than initially planned. Digestive periods examined were 4, 8, 16, 24, 28, and 32 h. For comparative purposes, we conducted the 24-h digestive period with both 2 and 3-fish meal sizes. Two northern pikeminnow were tested at each combination of digestive period and meal size. At the end of a digestive period, we inspected the tank for white sturgeon that had been regurgitated rather than retained

in the gut. Northern pikeminnow were killed by a blow to the head, and the entire digestive tract was removed and placed in a tray. The digestive tract was cut open and its contents examined with a dissecting microscope. The number of white sturgeon with intact bodies was counted, and a digestive index was assigned as follows: 1. White sturgeon in digestive tract easily recognizable; 2. Body intact, but not immediately recognizable. Further examination under a dissecting microscope reveals scutes, fins, and other body parts; 3. Digested material with scutes visible under a microscope; and 4. Digested material with no scutes apparent.

RESULTS

White sturgeon in northern pikeminnow digestive tracts were intact and recognizable up to about 16 h after force-feeding (Table 1). By 24 h, most white sturgeon bodies were not intact, but scutes and some body parts were still evident when contents were observed under a dissecting microscope. At 28 h, scutes remained undigested and were recognizable. By 32 h, very little material was present in the digestive tract, and no scutes were observed.

Some meals were smaller than initially planned in our study because of regurgitation and one northern pikeminnow had regurgitated the entire meal during the 32-h digestion period (Table 1). However, although digestion and evacuation rates for northern pikeminnow have been shown to be faster for smaller meals (Steigenberger and Larkin 1974, Beyer et al. 1988), this was not readily apparent. For example, both northern pikeminnow regurgitated one white sturgeon during the 28-h digestive period, resulting in a 1-fish meal each, and scutes were still identifiable (Table 1).

Table 1. Digestion of white sturgeon juveniles by 14 northern pikeminnow. Two northern pikeminnow were force-fed 2 or 3 white sturgeon at each digestion time—at 24 h, both numbers of prey were tested. Note that some meals were smaller than initially planned because white sturgeon were regurgitated. wst = white sturgeon.

Digestion time (h)	No. of wst per predator	No. wst regurgitated	No. wst intact in digestive tract	Digestive index
4	3	0	3	1
4	3	0	3	1
8	3	0	3	1
8	3	0	3	1
16	3	0	3	1
16	3	1	2	2
24	3	0	1	2
24	3	1	0	3
24	2	0	0	3
24	2	0	0	3
28	2	1	0	3
28	2	1	0	3
32	2	0	0	4
32	2	2	0	4

DISCUSSION

We found that juvenile white sturgeon could be observed in digestive tracts of northern pikeminnow for at least a day after feeding. If northern pikeminnow collected in past predation studies had been eating white sturgeon juveniles they probably would have been detected, since scutes remained in good condition in northern pikeminnow digestive tracts for 28 h, and small fish bones are commonly used to identify ingested fishes (Hansel et al. 1988). However, our results are not directly applicable to other common predators in the Columbia River Basin such as smallmouth bass, walleye, and channel catfish. These fishes have stomachs with acidic gastric juices which quickly digest bones, while northern pikeminnow have no stomachs and instead have an enlarged anterior limb of the intestine with a bile duct that enters the digestive tract directly behind the esophagus (Weisel 1962, Beyer et al. 1988). This system is less effective at bone digestion and, in fact, leaves even very small and delicate bones of fishes such as three-spine stickleback, *Gasterosteus aculeatus*, intact (C. Frost, personal observation).

The evacuation rates we found for white sturgeon were slower than those reported by Beyer et al. (1988) for northern pikeminnow feeding on juvenile chinook and coho salmon. They presented a relationship between evacuation time, temperature, and predator and prey weight. At 17°C for a 1000 g northern pikeminnow (about the mean weight of fish we used), meal sizes of 4, 8, and 12 g (approximate weights of 1, 2, and 3 white sturgeon in our study), would take 4.4, 6.8, and 8.7 h, respectively, for 90% evacuation. In contrast, white sturgeon were still intact after 8 h in northern pikeminnow digestive tracts, and roughly 90% evacuation did not occur until about a day had passed (Table 1).

There could be several causes for the slower evacuation rates we observed. Different prey types have been shown to digest at varying rates. For example, Singh-Renton and Bromley (1996) found that the gastric evacuation rate of brown shrimp, *Crangon vulgaris*, ingested by cod, *Gadus morhua*, and whiting, *Merlangius merlangus*, was much slower than that of herring, *Clupea harengus*, or sand eel, *Ammodytes* spp. This was likely because the exoskeleton of the shrimp delayed digestion—only after it had become softened and ruptured was the flesh digested. Perhaps the bony surface scutes and leathery skin on white sturgeon similarly slowed digestion.

Another factor for slower evacuation could have been that we force-fed our fish. Beyer et al. (1988) used natural feeding in her experiments, and theorized that force-feeding may result in an increased period of "lag time" before evacuation begins. However, Steigenberger and Larkin (1974) force-fed redside shiners, *Richardsonius balteatus*, to northern pikeminnow and generally found evacuation rates similar to Beyer et al. (1988). One result of force feeding may have been increased regurgitation of white sturgeon, although regurgitated prey have also been observed in laboratory experiments where northern pikeminnow were allowed to feed naturally on juvenile salmon (Gadomski and Hall-Griswald 1992).

Since we found that white sturgeon should be distinguishable in the digestive tracts of northern pikeminnow, there are a variety of possibilities why they have not been reported as a diet item for fishes in the Columbia River Basin. Many feeding studies have primarily collected predators in shallow waters where they are abundant and readily captured with electrofishing gear (Poe et al. 1991, Zimmerman 1999). Benthic white sturgeon juveniles generally occur in deep waters (Parsley et al. 1993, McCabe and Tracy 1994), and thus predators in these studies may have rarely encountered white sturgeon as prey. Furthermore, densities of small white sturgeon are low in many locations and vary annually (Parsley et al. 2002), so they may be infrequently eaten. To fully investigate if juvenile white sturgeon are preyed upon in the wild, digestive tract contents of benthic predators collected from deeper waters will need to be examined.

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PREDATION BY A GOLDEN EAGLE, *AQUILA CHRYSAETOS*, ON A JUVENILE MOUNTAIN SHEEP, *OVIS CANADENSIS*

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Golden eagles, *Aquila chrysaetos*, are large avian predators, and are one of few raptorial species capable of killing ungulates. Indeed, the literature contains numerous reports of golden eagles preying on a variety of large mammals (Mason 2000 for review), including the young of wild sheep, *Ovis canadensis*, *O. dalli* (Kennedy 1948, Nette et al. 1984). Female mountain sheep exhibit typical anti-predator behaviors when large avian scavengers are encountered (Stockwell 1991), suggesting that avian predators have been a more important selective force than heretofore recognized. Nevertheless, attacks on wild sheep by avian predators have been described infrequently and there is but one report (Nette et al. 1984) of young being carried off by an attacking eagle. Moreover, only one description of wounds received by wild sheep during attacks by golden eagles (Ober 1931) is available, and actual weights of individuals that were attacked have not been reported. In this note, we describe the successful attack by an

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adult golden eagle on a young mountain sheep, *O. canadensis*, and report necropsy results and actual weight of the prey.

At 1600 on 30 May 1996, we observed two adult golden eagles along the Snake River in Hells Canyon, the border between Oregon and Idaho. The eagles were soaring above the confluence of the river and Frenchy Creek, Nez Perce County, Idaho (45°53' N, 116°50' W). Simultaneously, we observed two female mountain sheep and a lamb, approximately 2 weeks-of-age, on a rocky outcrop. The eagles swooped low during alternating passes, and made several unsuccessful attempts to capture the lamb; one eagle then landed nearby (<100 m). One female sheep next ran approximately 20 m and, as the lamb attempted to follow, the second eagle stooped and captured the lamb. This eagle flew about 20 m from the point of capture and dropped the lamb, which fell about 50 m before striking the ground. The eagle immediately landed at the carcass, and we then climbed to that location to retrieve and examine the lamb.

As we approached, the eagle flushed off of the carcass. We examined the remains cursorily (compound fracture of a femur; protruding scapula; evidence of feeding by the eagle in the pelvic area) and collected nasal swabs to evaluate the possibility of disease exposure (see Cassirer et al. 1996). We then transported the carcass to the Idaho Department of Fish and Game Wildlife Health Laboratory, where additional swabs were obtained from the pulmonary tract for bacteriology. Necropsy revealed a male in good flesh (6.7 kg) with additional lesions including a fractured skull, fractures of both mandibles, and lacerated liver and left kidney. There was a large amount of hemorrhage in the peritoneal cavity and in the retro-peritoneal area. Those injuries are consistent with trauma that occurred when the lamb struck the ground. Laboratory culture of nasal and lung swabs yielded *Pasteurella multocida* identical to the organism associated with deaths of numerous mountain sheep after transfer to captivity from Hells Canyon, Washington (Cassirer et al. 1996).

The lamb described herein was in good condition; the stomach contained milk curd, the intestine contained normal ingesta, and necropsy yielded no physical abnormalities. Although subsequent investigations indicated that pneumonia is a primary cause of mortality of lambs in Hells Canyon during summer (Cassirer et al. 2001), there was no evidence that ability of the lamb to evade the eagle had been compromised by disease. Animals that are debilitated may be more susceptible to terrestrial predators than those that are not (Curio 1976). Whether that generality can be extended to include susceptibility to avian predators remains open to speculation, although Jonez (1961) described a lamb with an apparent congenital defect that may have predisposed it to predation by a golden eagle.

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THE OCCURRENCE OF THE JAPANESE MITTEN CRAB, *ERIOCHEIR JAPONICA* (DE HAAN), ON THE WEST COAST OF NORTH AMERICA

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In July 1997, a sturgeon angler using smelt for bait captured an adult male mitten crab (Fig. 1: carapace width 72.9 mm) in the Columbia River, approximately 3 km west of the Astoria-Megler Bridge in Astoria, Oregon. Although the Chinese mitten crab, *Eriocheir sinensis* H. Milne Edwards, had become well established in the San Francisco Estuary by this time (Cohen and Carlton 1997), this was the first U.S. west coast record of a mitten crab outside of California. Comparison of the specimen to *E. sinensis* collected in California revealed a number of morphological differences, suggesting that it could be a different species and therefore not derived from the California population.

Crabs of the genus *Eriocheir* are native to rivers and coastal areas of China, Korea, and Japan, and some are of considerable economic importance. Their taxonomy remains in flux, however, with various authors recognizing anywhere from three to five species

Figure 1. Dorsal view of male mitten crab captured from the Columbia River near Astoria, Oregon.

in as many as three genera (Chu et al. 2003). The debate concerning two of the species, *E. leptognathus* and *E. formosa*, centers only on whether one or both belong in the genus *Eriocheir* or in separate genera (Ng et al. 1999); their status as distinct species is not in doubt. Among other characters, the restriction of the setal "mittens" to the inner or outer face of the claws of *E. leptognatha* and *E. formosa*, respectively, readily differentiate them from all other mitten crabs.

The remaining three putative species, *E. sinensis*, *E. japonica*, and *E. hepuensis*, are more problematic. Li et al. (1993) concluded that all three are conspecific; however, there has been little support for combining *E. sinensis* and *E. japonica* due to perceived morphological (Chan et al. 1995, Montu et al. 1996, Guo et al. 1997) and genetic (Zhao et al. 2002, Chu et al. 2003) differences between the two. The third form, *E. hepuensis*, is morphologically intermediate between *E. sinensis* and *E. japonica*, and has variously been considered a hybrid, a distinct species, a subspecies of *E. japonica*, or a synonym of it. The close morphological and genetic similarities of the forms suggest a very recent radiation within the genus *Eriocheir* (Chu et al. 2003).

If the different forms represent separate species or subspecies, it could provide important insights into the source of introduced populations. Herein we will treat *E. sinensis* and *E. japonica* as distinct species for the sake of comparing them to the Columbia River specimen, and illustrate characters for their differentiation since existing keys (e.g., Sakai 1976; Dai and Yang 1991) are not readily available for many American workers. More importantly, the features used to differentiate the two species in existing keys are primarily subjective ("hairier", "flatter", "sharper" etc.) and this, coupled with a lack of figures, can make identification difficult when only one of the species is available. One notable exception is the paper by Chan et al. (1995), which provided photographs clearly illustrating the frontal teeth of the carapace of all five species and/or subspecies in the genus.

The Columbia River mitten crab was directly compared to specimens of *E. japonica* from Japan (3 males, 49.5 - 54.2 mm cw) and from Kyung Sang Nam Do, Sum-Jin estuary, Korea (2 males, 57.3-73.7 mm) along with *E. sinensis* from California (11 males, 72.1-88.5 mm) and Korea (2 males, 62.0-75.3 mm cw from Kyung Ki Do, Pa-Ju estuary). Although at least 12 different morphological characters have been cited as differentiating adult males of the two species, several of these proved too subtle or variable to be useful (e.g., shape of the telson and sixth abdominal somite; Guo et al. 1997). The following characters were found to differ consistently between the specimens examined that were identified as either *E. sinensis* or *E. japonica*. These are illustrated and discussed below in relation to the specimen from the Columbia River, which has been deposited at the California Academy of Sciences (CASIZ 168619).

Carapace convexity: The carapace of *E. sinensis* is reportedly more convex or domed than that of *E. japonica* (Sakai 1976, Dai and Yang 1991, Guo et al. 1997). To compare the relative convexity of the carapace of male *E. sinensis* and *E. japonica*, crabs were positioned so that the epistome was in the same plane as the posterior margin of the carapace in front of a stationary, level camera. When viewed from an anterior perspective, the carapace of *E. sinensis* (Fig. 2a) is noticeably more convex than that of *E. japonica* (Fig. 2b). This is due in part to the protogastric crest that is present in *E. sinensis* and

lacking in *E. japonica* (Chu et al. 2003), and the slightly inflated branchial region of the carapace in *E. sinensis*. The Columbia River specimen is distinctly flattened and lacks a protogastric crest (Fig. 2C).

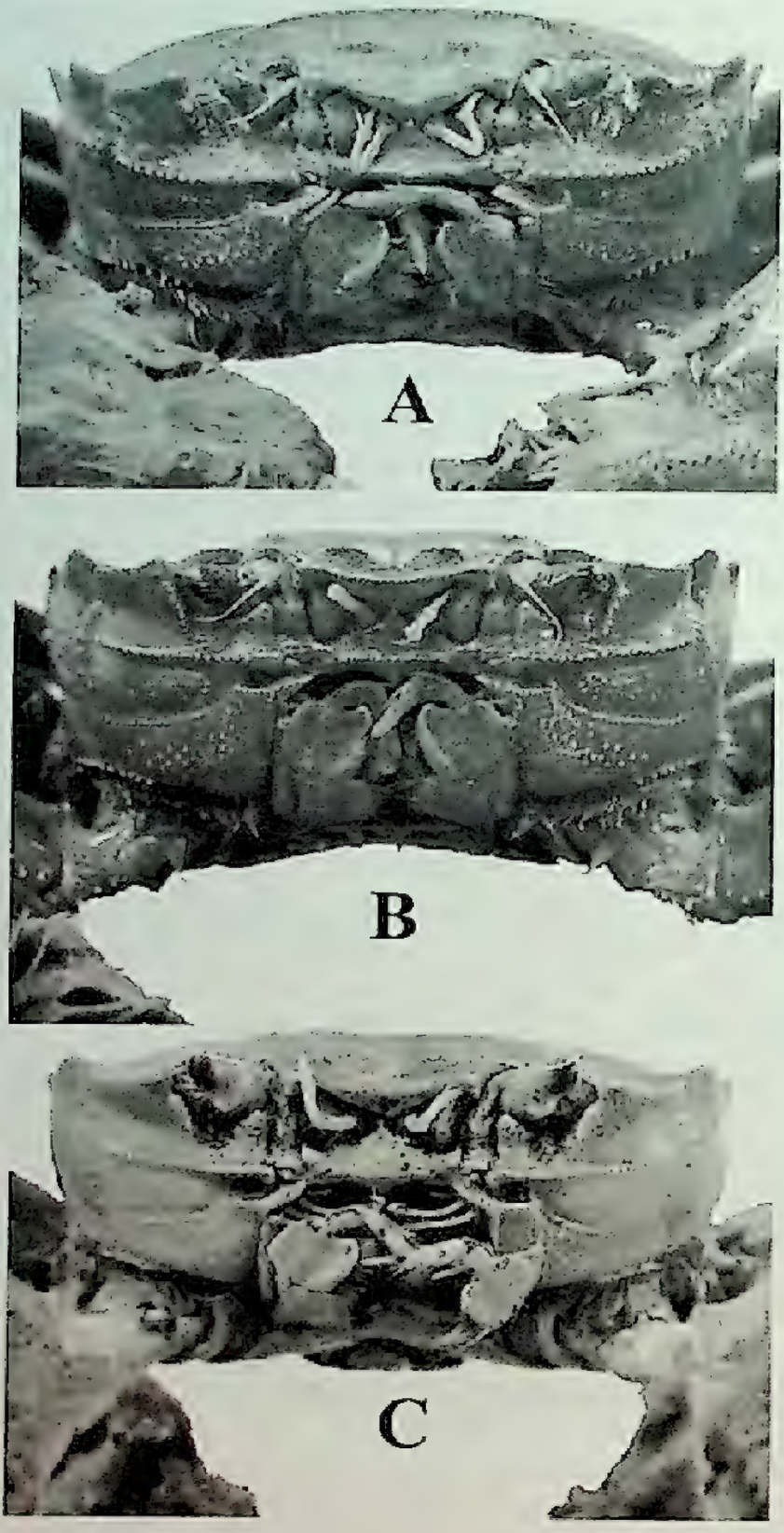

Figure 2. Anterior view of adult male mitten crabs. **A.** *Eriocheir sinensis* (75.3 mm CW) from Korea. **B.** *E. japonica* (73.7 mm CW) from Korea. **C.** Columbia River specimen.

Frontal margin: The frontal teeth of *E. sinensis* are sharper and more deeply divided than those of *E. japonica* (Sakai 1976). Although the length and acuteness of the frontal teeth of *E. sinensis* varied, even the specimen with the least development (Fig. 3a) had distinctly sharper and longer teeth than any of the *E. japonica* (Fig. 3b). The most conspicuous difference was in the depth of the median notch. The Columbia River specimen (Fig. 3c) has a shallow median notch and sinuous frontal margin typical of *E. japonica*.

Anterolateral spines: All four anterolateral teeth were very distinct on all the specimens of *E. sinensis* (Fig. 3a). The teeth of *E. japonica* (Fig. 3b) and the Columbia River specimen (Fig. 3c) were much less defined with the fourth tooth reduced to a tubercle, the only character that Chu et al. (2003) considered reliable for distinguishing *E. japonica* from *E. hepuensis*.

Fourth walking leg: The propodus of the last walking leg is reportedly narrow and slender in *E. sinensis* and flattened in *E. japonica* (Sakai 1976). The proportional width of the propodus was calculated by dividing the longest dimension of the segment (including the distal condyle, as shown in Guo et al. 1997) by the greatest width. Both

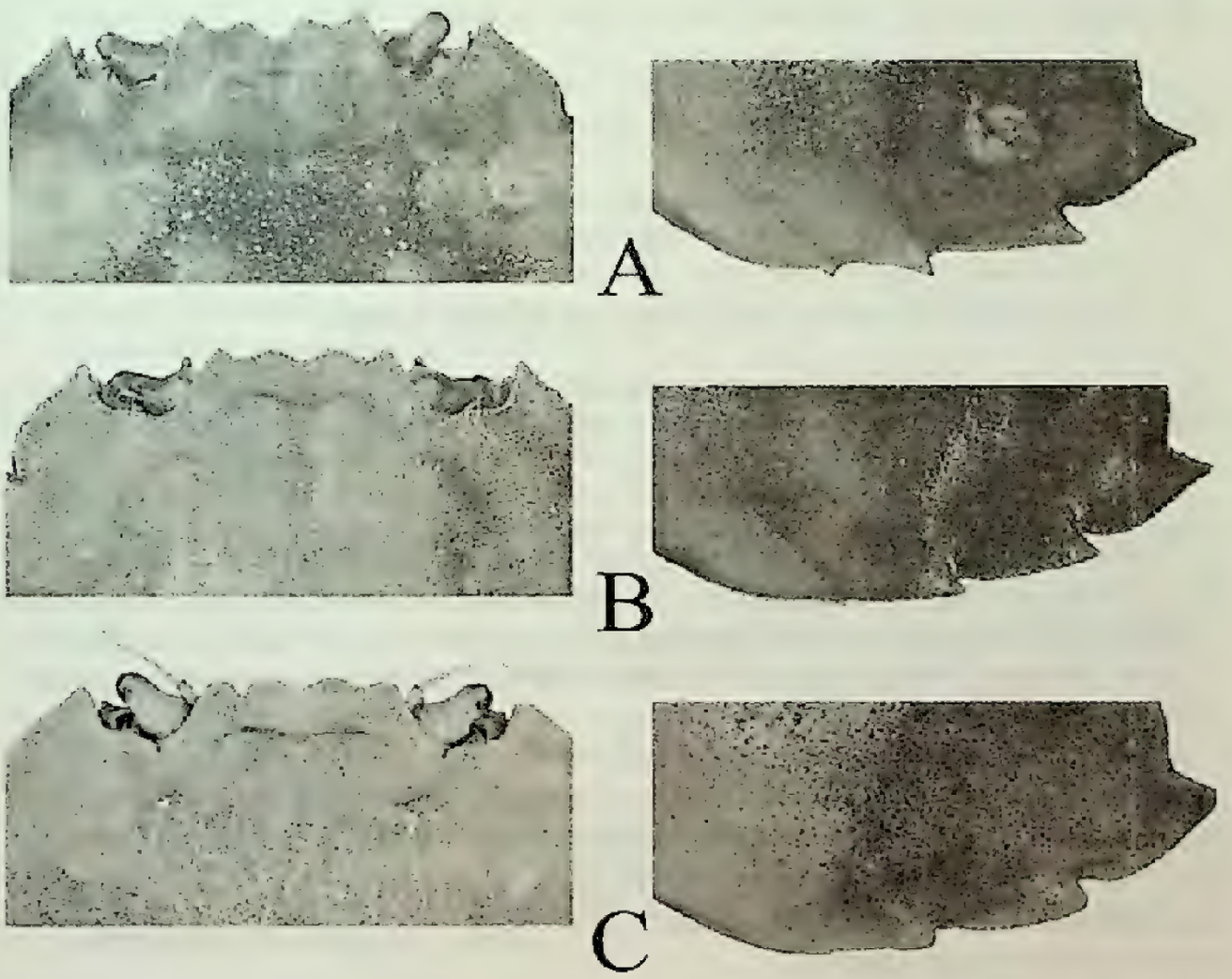

Figure 3. Frontal region and anterolateral carapace spines of adult male mitten crabs. **A.** *Eriocheir sinensis* (75.3 mm CW) from Korea. **B.** *E. japonica* (73.7 mm CW) from Korea. **C.** Columbia River specimen.

species showed high variability but minimal overlap, ranging from 2.7-3.5 for *E. sinensis* and 2.1-2.7 for *E. japonica* (Fig. 4). In the Columbia River specimen, the propodus was 2.5 times longer than wide.

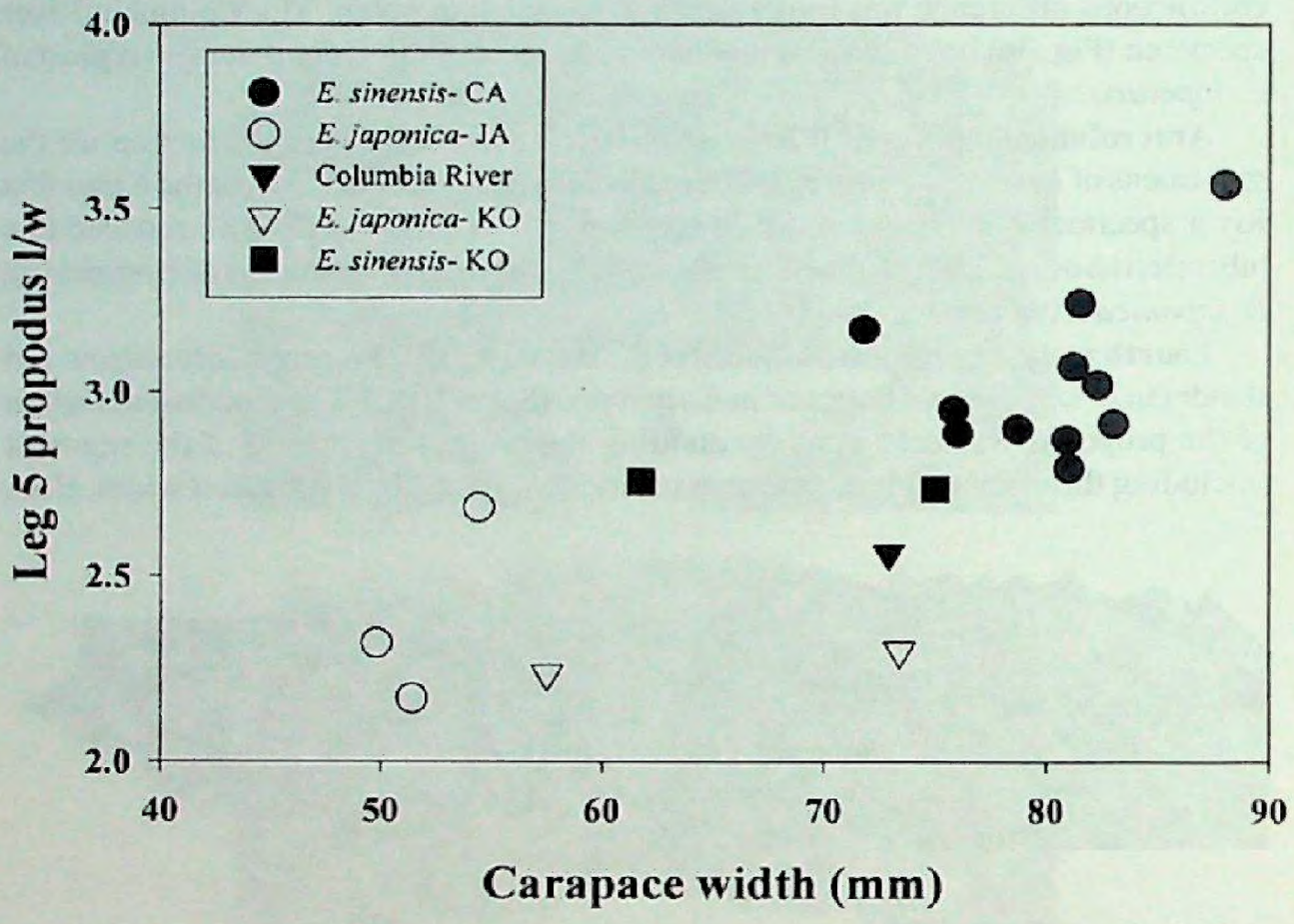

Figure 4. Proportional width of the propodus of the last walking leg of *Eriocheir sinensis* and *E. japonica* from California (CA), Korea (KO), Japan (JA), and the Columbia River.

Male gonopod 1: The genital pore is at the distal end of the first gonopod in *E. sinensis* and located subdistally in *E. japonica* (Guo et al. 1997; see Fig. 5 in Ng et al. 1999). The genital pore of the Columbia River specimen was subdistal like that of *E. japonica*, but as Chu et al. noted (2003), this is a difficult feature to examine and therefore not practical for use in the field.

In each of the characters that differed between large male specimens identified as *E. sinensis* or *E. japonica*, the Columbia River specimen matched the latter species. Since the San Francisco Bay specimens clearly match *E. sinensis*, this suggests that the Columbia River specimen was not derived from those in California, and represents both a separate mitten crab introduction to the west coast and the first record of *E. japonica* from North America.

The actual mechanism of its arrival is a matter of conjecture. Introduced copepods have become well established in the Columbia River estuary and were very likely introduced via discharges of ballast water from large ships (Cordell et al. 1992); it is

possible that the crab was transported as a planktonic larva in a similar manner. It is also possible that the animal was brought in and released, since live, illegally-imported adult mitten crabs have been intercepted on numerous occasions at Pacific coast airports (Cohen and Carlton 1997) and in some cases these may have been attempts to intentionally introduce the species to North America.

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